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13. ABSTRACT (Maximum 200 Words)

Prostate specific membrane antigen (PSMA) is the single most well-established, highly restricted prostate epithelial cell membrane antigen expressed by virtually all prostate cancers. PSMA is an ideal target for developing radiolabeled monoclonal antibodies (mAbs) for radioimmunotherapy (RIT) of prostate cancer. J591 mAb binds with very high affinity to the extra-cellular domain of PSMA and binds to viable tumor cells. We have previously submitted the final report of the Phase I of the Idea development Award in November 2000. Based on preclinical work, we proposed phase I dose-escalation RIT clinical trials in patients with prostate cancer, in order to study the safety and pharmacokinetics of ^{90}Y-DOTA-J591 labeled J591 (Phase II of the Idea development Award).

We have completed a phase I dose-escalation RIT trial in patient with prostate cancer (n=28). In this phase 2 final report, we documented that the maximum tolerated dose of ^{90}Y-DOTA-J591 mAbs is 17.5 mCi/m^2. The dose-limiting toxicity was myelotoxicity. Repeat administration of ^{90}Y-DOTA-J591 ($\leq$ 17.5 mCi/m^2) doses 2-3 months following the first treatment dose was also well tolerated. Administration of ^{90}Y-DOTA-J591 did produce significant anti-tumor response; either 70-85% declines in PSA lasting more than 6 months or PSA stabilization by week 12. Several patients had improvement in pain and performance status that did not necessarily correlate with PSA or measurable disease responses. There was strong concordance between PSA and measurable disease responses. We have clearly documented that ^{90}Y-DOTA-J591 mAb is a potential radiopharmaceutical for targeted RIT of prostate cancer.

14. SUBJECT TERMS
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INTRODUCTION

Prostate specific membrane antigen (PSMA) is the single most well-established, highly restricted prostate epithelial cell membrane antigen expressed by virtually all prostate cancers, and the expression is further increased in higher grade prostate cancers and in metastatic disease and in hormone-refractory prostate cancers. Therefore, PSMA is ideal for *in vivo* prostate specific targeting of radiolabeled antibodies. J591 monoclonal antibody binds with very high affinity to the extracellular domain of PSMA. In preclinical studies (Phase I of idea development research), we have demonstrated that J591mAb labeled with beta emitting radionuclides such as ^{131}I, ^{90}Y and ^{177}Lu are potentially useful for targeted radioimmunotherapy of prostate cancer since J591 mAb is specific to the extracellular domain of PSMA and binds to viable tumor cells. Based on preclinical work, we proposed phase I dose-escalation clinical trials in patients with prostate cancer, in order to study the safety and pharmacokinetics of ^{90}Y-DOTA-J591 labeled J591.

In the Phase II part of Idea Development Award, We **hypothesized that ^{90}Y-DOTA-huJ91 is an ideal radiopharma-ceutical for RIT** of metastatic prostate cancer. In addition, using **humanized mAb** (huJ591) would permit multiple injections of radiolabeled J591 antibody. The main objectives of the proposal were to perform a **Phase I dose escalation trial with ^{90}Y-DOTA-huJ91** and the specific aims were to 1) compare the safety and toxicity of **single doses vs. cumulative dose** (multiple administrations or re-treatment) of ^{90}Y-DOTA-huJ591. 2) define the pharmacokinetics and **radiation dosimetry** of ^{90}Y-DOTA-huJ591 using ^{111}In-DOTA-huJ591 blood kinetics and imaging studies. 3) define the safety, toxicity and maximum tolerated dose (**MTD**) of ^{90}Y-DOTA-huJ591.

In the Statement of Work (SOW) we described that the project could be accomplished in 4 phases (or steps). We have completed the trial as planned (or proposed). We requested extension of the project for one more year (with out additional funds) in order to complete all the data analysis and to finish the manuscripts for publication. By the end of year 2003, we have successfully completed all our goals of the Phase I and II of research proposal.

We have submitted the final report of the Phase I research in November 2000. This is the final report of the Phase 2 research of ideal development award.

Body: Research accomplishments

^{90}Y-DOTA-J591: Phase I Dose-Escalation Protocol

Dose preparation

J591-DOTA was supplied by BZL Biologics, Inc (Framingham, MA) under IND 9279. J591-DOTA was labeled with ^{111}In (MDS Nordion, Ontario, Canada) and ^{90}Y (Perkin-Elmer, Shelton, CT). The DOTA-J591 mAb was then labeled with ^{111}In or ^{90}Y chloride in an ammonium acetate buffer to produce specific activities of 111-222 MBq/mg (3-6 mCi/mg). Radiolabeled J591 was purified by gel filtration and sterilized by membrane filtration prior to administration into patients. Patients initially received a dose of ^{111}In (5 mCi)-J591 for PK and biodistribution determinations. One week later, patients received ^{90}Y-J591 treatment dose as per the dose-escalation strategy. With both ^{111}In and ^{90}Y doses, all patients received a total of 20 mg of mAb J591. All mAb administrations were by intravenous infusion at $\leq$ 5 mg/min.

Patient Eligibility, Screening

Eligible patients had a prior histologic diagnosis of prostate cancer with evidence of recurrent or metastatic disease as defined by a rising PSA and/or abnormal radiologic studies including bone scan, computed axial tomography (CT) and/or magnetic resonance imaging (MRI). Patients were required to have a PSA $\geq$ 1.0 at study entry with three consecutive rising PSA values over a period of $\geq$ 2 weeks. Eligible patients were > 21 years of age with a KPS of at least 60% and life expectancy > 6 months at the time of entry. Pretreatment evaluation included a history, physical examination, routine laboratory studies including PSA, prostatic acid phosphatase (PAP), testosterone and an electrocardiogram. Radiological studies included a chest x-ray, CT or MRI of the abdomen, pelvis and brain as well as a bone scan. Patients were required to have a bone marrow biopsy within six weeks of study entry.

Biodistribution and Dosimetric Studies with ^{111}In-J591 Each patient received a diagnostic dose of 5 mCi of ^{111}In-J591 (1-2 mg) mixed with cold J591 mAb mass (18-19 mg) in a total volume of 20 mL. Venous blood samples (4 mL) were obtained at 10 minutes, 1, 2, 4, and 24 hours and days 2, 3, 4 and 7. The percent injected dose (% I.D.) was determined by measuring an aliquot of blood along with a known ^{111}In standard.

In order to assess the biodistribution of mAb J591, total body gamma camera images were obtained within 1 hour post-infusion (day 0) and again at 4 additional time points in the subsequent week (e.g. days 1, 2, 3 and 6-7). The gamma camera images were obtained using a dual head ADAC gamma camera fitted with an appropriate collimator (ADAC, Milpitas, CA or GE, Inc., Milwaukee, WI). The % I.D. in major organs (heart, liver, spleen, kidneys, bone marrow, GI tract and bladder) was estimated by drawing regions of interest (ROI) and determining the relative counts in each organ and kinetics of wash out from each organ. SPECT studies of the abdomen, pelvis and/or areas of suspected metastatic lesions were performed on day 2 or 3 in selected patients. Based on PK and imaging studies with ^{111}In-J591, radiation dosimetry of ^{90}Y-J591 was estimated.

^{90}Y-J591 Dose-Escalation Protocol

Following completion of 111In studies, each patient received ^{90}Y dose, which was escalated in

cohorts of 3 to 6 patients at the following planned dose levels: 5, 10, 15, and 20 mCi/m^2. A fifth dose level of 17.5 mCi/m^2 was added to more precisely define the MTD. Dose escalation was held until at least 3 patients at each dose level had been followed for 6 weeks without evidence of hematologic toxicity. If any of the initial 3 patients at a dose level experienced grade 1 or 2 hematologic toxicity by 6 weeks, dose escalation was held until the onset of blood count recovery was demonstrated. If any patient experienced grade 3 or 4 hematologic toxicity, at least 6 patients were entered at that dose level and followed until onset of blood count recovery prior to dose escalation. If, at any time, 2 instances of DLT were observed at a given dose level, further entry at that dose level was terminated. Patients were followed for a minimum of 12 weeks after ^{90}Y-J591 administration. Routine clinical and laboratory assessments (including biochemical profile, PSA, PAP and testosterone) were performed at defined intervals. Complete blood count (CBC) and platelet counts were initially monitored 1-2 times per week and then every 4 weeks until blood count stabilization.

Re-Treatment

Patients were considered eligible for re-treatments with ^{90}Y-J591 at ≥ 6 week intervals if their platelet and neutrophil count recovery was satisfactory (platelet count ≥ 70% of the baseline platelet count of the prior, most recent treatment cycle with a minimum recovery to at least 75 x 10^9/L; and ANC was ≥ 80% of the baseline ANC of the prior, most recent treatment cycle with a minimum recovery to 1.3 x 10^9/L). Patients who experienced any ≥ grade 3 non-hematologic toxicity in a prior ^{90}Y-J591 treatment cycle were ineligible for re-treatment. Patients were followed for a minimum of 12 weeks after their last dose of ^{90}Y-J591 and those patients with stable or responding disease followed until progression.

Dose limiting toxicity (DLT) was defined as the following: *Hematologic toxicity* consisting of grade 4 thrombocytopenia (platelet < 10 x 10^9/L) and/or grade 4 neutropenia (ANC < 0.5 x 10^9) for >5 days; and *other toxicity* consisting of any grade ≥ 3 non-hematologic toxicity attributable to ^{90}Y-J591.

Maximum tolerated dose (MTD) was defined as the dose level at which 0/6 or 1/6 patients experience a DLT with the next higher dose level having ≥ 2 patients of 6 experiencing DLT. Once the MTD was reached, at least 6 patients were to be evaluated at that dose level.

Tumor Assessments

Response was assessed either biochemically (PSA change) and/or by change in size of measurable lesions. Biochemical response was determined by comparing the nadir PSA level after treatment to the PSA determined immediately prior to initiating therapy. PSA response was defined as a > 50% decrease from baseline maintained for at least 4 weeks.[30] Biochemical (PSA) progression was defined as a ≥ 25% rise in PSA above the baseline, pre-treatment value.

In patients with measurable disease, the following definitions were used: Complete response was the complete disappearance of all measurable lesions by physical examination or imaging studies with no appearance of new lesions for ≥ 2 months; partial response was defined as a ≥ 50% decline in the sum of the products of the longest perpendicular diameters of all measurable lesions without the development of new lesions; stable disease occurred in patients who did not

meet the criteria for a partial response and who were without signs of progressive disease for ≥ 2 months; and progressive disease was defined as a ≥ 25% increase in the sum of the products of the longest perpendicular diameters of the indicator lesions or the appearance of new lesions.

Duration of response was the time interval from treatment initiation until progression as documented by either a rise in PSA, enlargement of a measurable lesion(s), or new lesion(s) on bone scan. The rising PSA was confirmed by a second, serially rising PSA and the duration defined as the time from initiation of treatment to the time of the first rising PSA.

Results and Discussion

Twenty-nine eligible patients (**Table 1**) with advanced PC were enrolled in the study between October 2000 and May 2002. One patient had a history of venous thrombosis and died of a probable pulmonary embolus after self-discontinuing his warfarin. Based on the nature of this death, this patient was considered not evaluable. Therefore, a total of 28 patients completed the protocol. The ^{90}Y-J591 dose escalation scheme and the numbers of patients treated at each dose level and number of re-treated patients are listed in **Table 2.**

Three patients received a second dose of ^{90}Y-J591: two patients at 17.5 mCi/m^2 with and one patient at 20 mCi/m^2. A single patient at the 17.5 mCi/m^2 dose level received three ^{90}Y-J591 doses.

Hematologic Toxicity

All hematologic toxicity is summarized in **Table 3**. At the 20 mCi/m^2 dose level, after two patients developed grade 3 thrombocytopenia with non-life threatening bleeding episodes, requiring platelet transfusions, accrual was ended after a total of 4 patients. Also at this dose level, one patient developed grade 4 neutropenia Although this was not the pre-defined DLT, these events were considered to be dose limiting. The 17.5 mCi/m^2 dose level was added to better define the MTD. A total of six patients were treated at this dose level with no DLTs. The median time to platelet nadir was day 28 and the median time to ANC nadir was day 35. The median time to platelet recovery (> 150 x 10^9/L) was day 49 and the median time to ANC recovery (> 2.0 x 10^9/L) was day 63. With a single dose, full platelet recovery (> 150 x 10^9/L) and full ANC recovery (> 2.0 x 10^9/L) was seen in 90% of the patients.

The three patients who received second treatment and one patient who received 3 treatments experienced grade 3 thrombocytopenia and neutropenia; one patient at 20 mCi/m^2 experienced grade 3 thrombocytopenia and neutropenia. **No DLTs were seen in the re-treated patients.**

Non-hematologic Toxicity

Non-hematologic toxicity was only mild or moderate and not dose-limiting. The majority of episodes were grade 1 and limited to fatigue, anorexia, nausea and mild transaminitis. Four of the eleven episodes of grade 1-2 AST/ALT elevations had elevated transaminase levels at baseline. One patient experienced an upper extremity venous thrombosis related to a central catheter. No dose limiting non-hematolgic toxicity was seen in the four re-treated patients. There was no evidence of HAHA (human anti-human antibodies) in any of the patients entered onto this trial.

Antitumor Activity

Two patients at the 20 mCi/m^2 dose level experienced 85% and 70% declines in PSA lasting more than 6 months (**Figure 1**). In addition, these two patients had objective measurable disease responses with a 90% and 40% decrease in the size of pelvic and retroperitoneal lymphadenopathy. Both patients were hormone-refractory with lymph node only disease and had not received prior chemotherapy. The second patient was **re-treated** with ^{90}Y-J591 on day 119. An additional 6 patients experienced PSA stabilization by week 12. Several patients had improvement in pain and performance status that did not necessarily correlate with PSA or measurable disease responses.

There was strong concordance between PSA and measurable disease responses (**Table 4**). Of 13 patients with soft tissue disease, 12 had measurable disease and 6/12 demonstrated progression, 4 stable disease and 2 had major objective responses. In 9/12 cases, PSA response agreed with the measurable observation. In 2 cases, PSA progressed while measurable disease was stable indicating the greater sensitivity of PSA. In one case PSA was stable while measurable disease progresses.

J591 Targeting

Gamma camera images of ^{111}In-J591 obtained on days 3-6 showed very specific targeting of radiolabeled J591 mAb in metastatic prostate cancer sites (**Figure 2**). Among the 29 patients who received ^{111}In-J591, 19 patients had bone lesions and 13 patients had soft tissue lesions. 17/19 (89%) patients with bone lesions and 9/13 (69%) patients with soft tissue lesions were accurately targeted resulting in an overall targeting sensitivity of 26 of 32 (81%) (**Table 5**). No false positive ^{111}In-J591 scans occurred.

Pharmacokinetics (PK) and Biodistribution of ^{111}In-J591

Based on plasma-time activity data and mono-exponential curve fitting, mAb ^{111}In-J591 cleared from circulation with a half-life of 32±8 hours (**Table 6**). The volume of distribution of the radiolabeled J591 antibody was estimated to be 4467±811 mL with a clearance rate of 98±43 mL/hr. The bi-exponential curve fitting of plasma-time activity data showed that more than 80% of labeled antibody clears from circulation with a half-life (β component) of 44±14 hr. The imaging studies have clearly documented that most of the activity was initially in the circulation and the only organ sequestering a significant amount of ^{111}In activity was the liver. By day 6, almost 70% of the injected dose was still remaining in the whole body with the liver uptake comprising 28±8% of the injected dose.

Radiation dosimetry of ^{90}Y-J591

Based on PK and imaging data with ^{111}In-J591, the radiation dosimetry of ^{90}Y-J591 was estimated and results summarized in **Table 7**. The critical organ with highest radiation dose is liver (24±8 rads/mCi), followed by spleen and kidneys. The radiation dose to bone marrow based on blood radioactivity is 3.4±1.6 rads/mCi of ^{90}Y dose administered.

Table 1 – Baseline Patient Characteristics

Characteristics	**No. of Patients (n = 29)**
Median age (range)	71 (50–85)
Median PSA μg/L (range)	64.8 (1.8-1918)
Median Alkaline phosphatase (range)	93 (56-694)
Median hemoglobin g/liter (range)	12.5 (9.8-15.9)
Median WBC x 10^9/L (range)	5.8 (3.0-9.5)
Median ANC x 10^9/L (range)	3.6 (2.1-11.1)
Median platelet count x 10^9/L (range)	227 (148*-453)
Primary Local Treatment	
Radical prostatectomy (RP)	12
Radiation therapy	6
Cryosurgery	1
None	10
Sites of Metastases	
Bone	19
Soft tissue	13
Prior therapy	
Chemotherapy	11
Radiation therapy (to bone)	8
Post-RP Radiation (to prostate)	8

Table 2: Summary of Dose Escalation Scheme

Dose level	mAb ^{90}Y-J591 (mCi/m^2)	No. of patients	Re-treated patients
1	5	4	0
2	10	7	0
3	15	8	0
4	17.5	6	3
5	20	4	1

Table 3: Hematological Toxicity: Summary

Dose	Patients	Thrombocytopenia, Grade					Neutropenia, Grade				
mCi/m^2	(n)	0	1	2	3	4	0	1	2	3	4
5	4	-	3	1	-	-	-	3	1	-	-
10	7	-	2	2	3	-	2	1	1	3	-
15	7	-	3	2	2	-	3	1	1	2	-
17.5	6	-	1	1	4	-	1	-	-	5	-
20	4	-	1	-	1	2	1	-	1	2	1

Table 4 – Correlation of PSA and Measurable Disease Response

	Progression	Stable	Response
PSA Progression	5	2	0
PSA Stable	1	2	0
PSA Response	0	0	2

Table 5: Plasma Clearance Kinetics: ^{111}In-J591 vs. ^{177}Lu-J591

PK parameter	^{111}In-J591 Plasma Clearance	
	Bi-exponential	Mono-exponential
$T_{½}$ (hr)	-	32.3 ± 8.1
α	2.37 ± 1.94	-
B (terminal)	44.2 ± 13.9	-
Area under the curve (AUC)	1.19 ± 0.44	1.08 ± 0.4
Volume of Distribution, V_d at T_0	4042 ± 863	4467 ± 811
Clearance (mL/hr)	94 ± 34	98 ± 43

Table 6: J591 Targeting Compared with Conventional Imaging

Metastatic Sites	^{111}In-J591 Scan Positive	Conventional Imaging	% positive ^{111}In-J591 Imaging
Bone	17	19	17/19 (89%)
Soft Tissue	9	13	9/13 (69%)
Bone and/or Soft Tissue	26	32	26/32 (81%)

Table 7 - Radiation Dosimetry of ^{111}In-J591 and ^{90}Y-J591

Organ	Radiation Dosimetry (cGy/mCi)	
	^{111}In-DOTA-J591	^{90}Y-DOTA-J591
Liver	4.23 ± 3.30	24.39 ± 8.39
Spleen	2.61 ± 3.24	18.22 ± 6.14
Kidneys	2.51 ± 2.82	16.55 ± 4.00
Heart Wall	2.11 ± 2.46	11.07 ± 2.24
Lungs	1.72 ± 2.43	10.63 ± 2.62
Red Marrow	0.72 ± 0.83	3.37 ± 1.59
Bone Surfaces	0.74 ± 0.57	2.53 ± 0.97
Urin. Bladder Wall	0.51 ± 0.22	2.63 ± 1.09
Muscle	0.40 ± 0.21	1.08 ± 0.28
Testes	0.27 ± 0.23	1.08 ± 0.28
Total Body	0.55 ± 0.36	2.05 ± 0.31

Figure 1: **PSA graphs for two patients at the 20 mCi/m^2 dose level demonstrating 80% decline in PSA after 1st treatment. After 2 months, PSA levels were gradually increasing. At 3 months, the 2nd treatment also showed drop and subsequent stabilization of PSA lasting almost 8 months.**

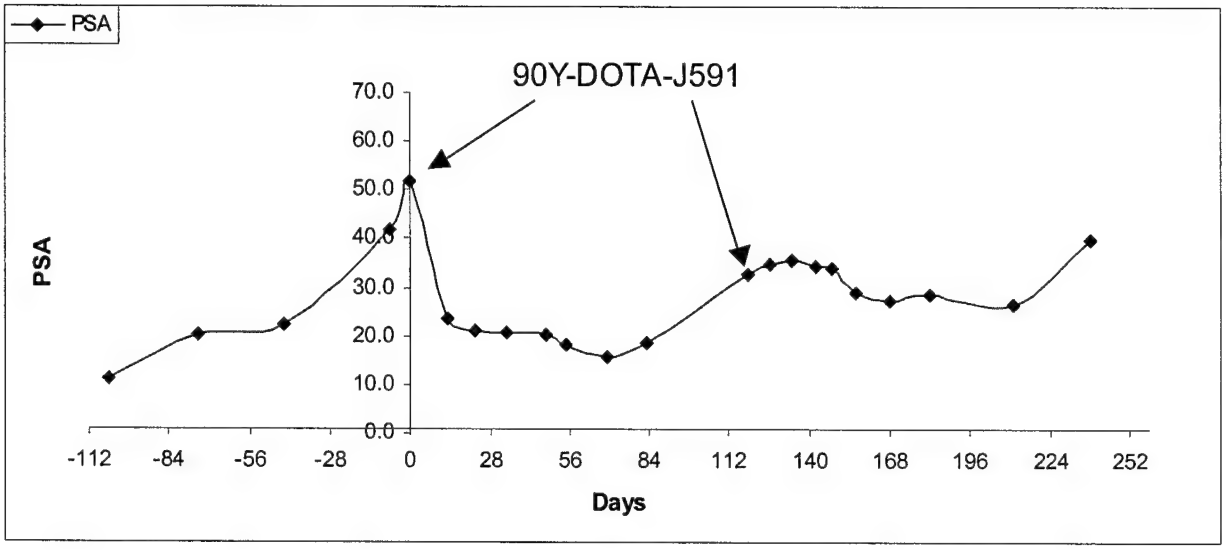
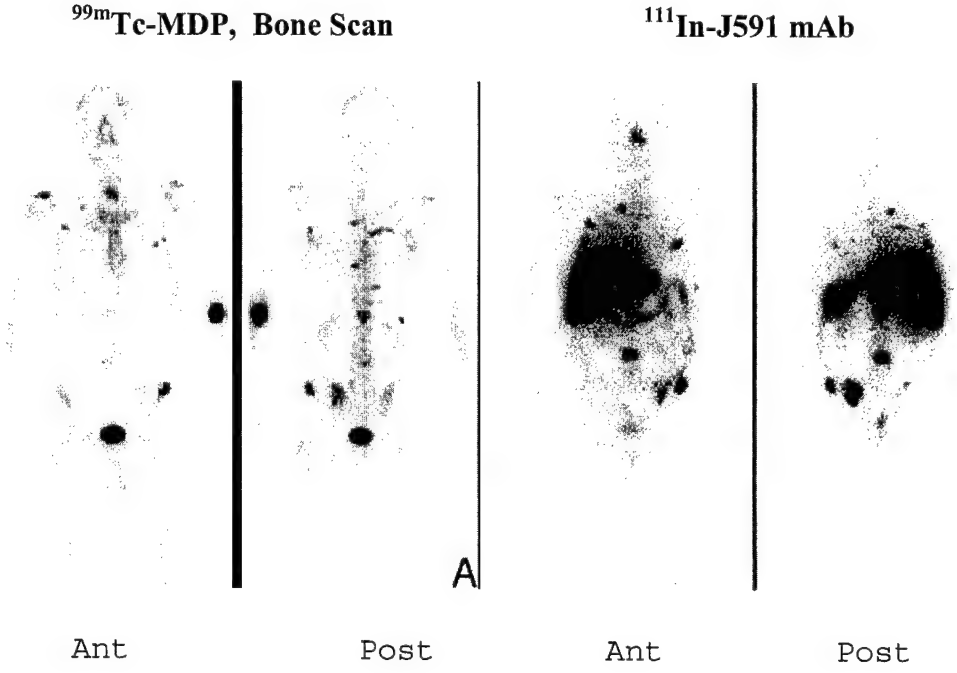


Figure 2 – Bone scan with corresponding ^{111}In-J591 mAb images in a patient with metastatic prostate cancer



Key Research accomplishments

1. Based on imaging studies in 29 human subjects with ^{111}In-DOTA-J591 mAb we have clearly demonstrated that radiolabeled de-immunized mAb targets specifically and sensitively metastatic sites in patients with hormone refractory prostate cancer.

2. Following administration of radiolabeled J591 mAb, the blood clearance of the antibody can be described predominantly based on bi-exponential curve fitting. More than 80% of the antibody clears from the circulation with a half-life (β component) of 44±14 hr.

3. We performed a Phase I dose-escalation radioimmunotherapy (RIT) trial with ^{90}Y-DOTA-J591 mAb in patients with prostate cancer (n=28). We have determined that the maximum tolerated dose (MTD) of ^{90}Y-DOTA-J591 mAb is 17.5 mCi/m^2.

4. We have demonstrated that the administration of ^{90}Y-DOTA-J591 mAb doses $\leq$17.5 mCi/m^2 is well tolerated by patients. The dose-limiting toxicity was myelotoxicity.

5. In a limited number of patients (n=4), we have also demonstrated that repeat administration of ^{90}Y-DOTA-J591 mAb doses $\leq$17.5 mCi/m^2, 2-3 months following the first treatment dose was also well tolerated.

6. Administration of ^{90}Y-DOTA-J591 did produce significant anti-tumor response. 2/4 patients at the 20 mCi/m^2 dose level experienced 70 - 85% declines in PSA lasting more than 6 months. An additional 6 patients experienced PSA stabilization by week 12. Several patients had improvement in pain and performance status that did not necessarily correlate with PSA or measurable disease responses. There was strong concordance between PSA and measurable disease responses.

Note:

We have recently completed (not part of Idea Development Award) a Phase 1 dose-escalation RIT trial with ^{177}Lu-DOTA-J591 mAb in patients with prostate cancer. The MTD with ^{177}Lu-DOTA-J591 was 70 mCi/m^2.

Reportable outcomes and Bibliography

Phase 1 part of Idea Development Award (June 1998 – November 2000)

We have previously submitted the "final report" of phase 1 part of this award in November 2000. This is an update on reportable outcomes

Manuscripts

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Phase 2 part of Idea Development Award (December 2000 – November 2003)

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Conclusions

Prostate specific membrane antigen (PSMA) is the single most well-established, highly restricted prostate epithelial cell membrane antigen expressed by virtually all prostate cancers. PSMA is an ideal target for developing radiolabeled monoclonal antibodies (mAbs) for radioimmunotherapy (RIT) of prostate cancer. J591 mAb binds with very high affinity to the extra-cellular domain of PSMA and binds to viable tumor cells. In preclinical studies (Phase I of idea development research), we have demonstrated that J591mAb labeled with beta emitting radionuclides such as ^{131}I, ^{90}Y and ^{177}Lu are potentially useful for targeted radioimmunotherapy of prostate cancer. Based on preclinical work, we proposed phase I dose-escalation RIT clinical trials in patients with prostate cancer, in order to study the safety and pharmacokinetics of ^{90}Y-DOTA-J591 labeled J591.

In this phase 2 final report, we documented that the maximum tolerated dose of ^{90}Y-DOTA-J591 mAbs is 17.5 mCi/m^2. The dose-limiting toxicity was myelotoxicity. Repeat administration of ^{90}Y-DOTA-J591 ($\leq$ 17.5 mCi/m^2) doses 2-3 months following the first treatment dose was also well tolerated. Administration of ^{90}Y-DOTA-J591 did produce significant anti-tumor response; either 70-85% declines in PSA lasting more than 6 months or PSA stabilization by week 12. Several patients had improvement in pain and performance status that did not necessarily correlate with PSA or measurable disease responses. There was strong concordance between PSA and measurable disease responses. We have clearly documented that ^{90}Y-DOTA-J591 mAb is a potential radiopharmaceutical for targeted RIT of prostate cancer.

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Radioimmunotherapy of Prostate Cancer in Human Xenografts Using Monoclonal Antibodies Specific to Prostate Specific Membrane Antigen (PSMA): Studies in Nude Mice

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BACKGROUND. Prostate specific membrane antigen (PSMA), expressed by virtually all prostate cancers is an ideal target for targeted therapy of prostate cancer. Radiolabeled J591 monoclonal antibody (MAb) binds with high affinity to an extracellular epitope of PSMA and localizes specifically in PSMA positive LNCaP tumors in vivo.

METHODS. Pre-clinical radioimmunotherapy (RIT) studies using ^{131}I-huJ591 and ^{90}Y-1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid (DOTA)-huJ591 MAbs were studied in nude mice bearing LNCaP xenografts.

RESULTS. A 15–90% reduction in mean tumor volume was observed after a single dose of ^{131}I-huJ591 (3.7–11.1 MBq) or ^{90}Y-DOTA-huJ591 (3.7–7.4 MBq). The median survival time increased 2–3 times relative to untreated controls. Multiple administrations of fractionated doses of ^{90}Y-DOTA-huJ591 were even more effective with minimal toxicity. Radiation dose to blood and tumor was higher with ^{90}Y than with ^{131}I. The maximum tolerated dose (MTD) is 5.55 MBq for ^{90}Y-DOTA-huJ591 and more than 11.1 MBq for ^{131}I-huJ591. For ^{90}Y-DOTA-huJ591 at MTD, dose to the tumor was 2,753 cGy.

CONCLUSIONS. In nude mice bearing PSMA positive tumors, radiation dose to the tumor with ^{90}Y-DOTA-J591 is greater for large tumors than with ^{131}I-J591. The theoretical and practical considerations strongly suggest that ^{90}Y-DOTA-huJ591 may be a suitable radiopharmaceutical for the treatment of prostate cancer. *Prostate 58: 145–155, 2004.* © 2003 Wiley-Liss, Inc.

KEY WORDS: **anti-PSMA antibody; prostate specific membrane antigen (PSMA); ^{131}I-huJ591 MAb; ^{90}Y-huJ591 MAb; LNCaP xenografts**

Shankar Vallabhajosula and Peter M. Smith-Jones contributed equally to this study.

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INTRODUCTION

Radioimmunotherapy (RIT) using monoclonal antibodies (MAbs) is undergoing investigation in many tumor types because of its ability to specifically target tumor sites while sparing normal tissues. A number of murine, chimeric or humanized MAbs, or their fragments, labeled with α, β- or Auger electron emitting radionuclides have been developed to target tumor related or associated antigens [1–3]. ^{90}Y and ^{131}I labeled anti-B1 MAbs for the treatment of lymphoma have shown 40–70% anti-tumor response in patients and are FDA approved [4,5].

Metastatic prostate cancer is a rationale candidate for RIT. Serum prostate specific antigen (PSA) monitoring can signal progression and/or recurrence of disease years before disease is detectable on imaging studies and additional years before clinical failure thereby providing the opportunity to identify and treat patients with microscopic disease burdens. Furthermore, prostate cancer is radioresponsive, often manifests as numerous small-volume sites of metastatic disease that receive high levels of antibody [6] (e.g., lymph nodes, marrow), and expresses prostate specific membrane antigen (PSMA). The latter is crucial, as PSMA is the single most well-established, highly restricted, prostate epithelial cell membrane antigen [7–14]. In contrast to other highly restricted prostate-related antigens such as PSA and prostatic acid phosphatase (PAP), both of which are secretory proteins, PSMA is anchored to the cell membrane [7]. The *PSMA* gene has been cloned, sequenced [15], and mapped to chromosome 11 [16]. Among reasons for significant interest in PSMA is that it is ideal for in vivo prostate-specific targeting strategies. In addition to its prostate specificity, PSMA is expressed by virtually all prostate cancers [12,13,17], expression progressively increases in higher grade cancers, in metastatic disease [12] and in hormone-refractory prostate cancers [11–13]. Given the theoretical advantages of MAbs in prostate cancer, and PSMA as an in vivo target for cell killing, it is compelling to evaluate this approach to develop anti-PSMA MAbs labeled with radionuclides or cytotoxic agents for the treatment of prostate cancer.

Initial validation of PSMA as an in vivo target has been borne out by imaging trials with MAb 7E11/CYT-356. A DTPA conjugated form of the 7E11/CYT-356 (Capromab Pendetide) that can be radiolabeled with ^{111}In, is commercially available (ProstaScint®) and FDA approved for diagnostic imaging of prostatic fossa recurrence and/or lymph node metastasis [18–20]. Molecular mapping, however, indicates that MAb 7E11/CYT-356 targets a cytoplasmic epitope of the PSMA molecule that is not exposed on the outer cell surface [20,21]. In viable cells, this internal epitope is not accessible to antibody and successful imaging with ProstaScint® relates to targeting only of dead/dying cells within tumor sites [22,23]. It has been predicted that a MAb to the extracellular domain of PSMA would provide benefits including improved localization in patients and enhanced imaging and therapy [22–24].

J591 is an IgG MAb with a high affinity for PSMA. J591 specifically binds to the external domain of PSMA ($PSMA_{ext}$) [22] and is rapidly internalized [23,24]. J591 demonstrates high affinity binding to viable prostate cancer cells in tissue culture, on tissue sections, and in animal models in vivo. Furthermore, unlike ProstaScint®, J591 can bind to viable cells, as the target binding site is present on the exterior of the cell [22]. Using genetic engineering techniques, the mouse (muJ591) antibody has been "deimmunized" by replacing murine protein sequences with human sequences. As a result, the humanized J591 (huJ591) MAb can be administered to patients on multiple occasions over long time periods without inducing an immune response.

In order to bind radiometals such as ^{111}In and ^{90}Y to the MAb, we have first conjugated a macrocyclic chelating agent, 1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid (DOTA) to MAbs J591 and J415 [25]. Based on in vitro studies, we have reported previously that ^{131}I-huJ591, ^{111}In-DOTA-huJ591, and ^{111}In-DOTA-muJ415 recognize and bind with high affinity to PSMA positive LNCaP tumor cells in vitro [25]. In addition, in nude mice bearing LNCaP tumors, both ^{131}I and ^{111}In labeled J591 and J415 (a murine MAb with high affinity for PSMA) recognize and bind with high affinity to PSMA-positive LNCaP tumor cells. In addition, in nude mice bearing LNCaP tumors, both ^{131}I and ^{111}In labeled J591 and J415 showed specific tumor localization [26]. The in vitro and in vivo studies suggest that anti-$PSMA_{ext}$ MAb J591 labeled with β-emitting radionuclides (^{90}Y and ^{131}I) would be potentially useful for targeted RIT of prostate cancer.

Taken together, these investigations have thus demonstrated that deimmunized J591 can be successfully conjugated to a radioisotope and be delivered to its biologic target with high specificity and potential anti-tumor activity. Herein, we report the pre-clinical RIT efficacy studies comparing the in vivo anti-tumor and dose–response relationships of ^{131}I and ^{90}Y labeled huJ591 MAb preparations in nude mice bearing LNCaP xenografts.

MATERIALS AND METHODS

All reagents were obtained from commercial sources. ^{131}I as sodium iodide was purchased from

Nordion (Kanata, Ontario, Canada) and ^{90}Y as yttrium chloride was purchased from Perkin-Elmer (Boston, MA). In order to reduce metallic contamination, all the reagents used to modify and purify the monoclonal antibodies were made with deionized water. Ammonium acetate buffer and sodium phosphate buffer were purified using Chelex 100 anionic resin (Bio-Rad, Richmond, CA) to remove any metal ions. Murine MAb J591 was initially prepared as described earlier [22]. Using genetic engineering techniques, the mouse (muJ591) antibody has been "deimmunized" by replacing murine protein sequences with human sequence and was supplied as a sterile and pyrogen-free huJ591 MAb preparation at a concentration of 5 mg/ml (BZL Biologics, Boston, MA). Two irrelevant IgG antibodies, anti-CD-20 murine MAb (IA-1) and F23 anti-renal cancer MAb (IA-2) were used as control antibodies in RIT studies.

Preparation of Radiolabeled Monoclonal Antibodies

huJ591 was labeled with ^{131}I using iodogen method to a specific activity of 400 MBq/mg (10.8 mCi/mg) as previously described [25]. In order to label huJ591 with ^{90}Y, the antibody was first conjugated with DOTA, by direct coupling of one of the four carboxylic acid groups of DOTA to the primary amines in the antibody protein structure [25,27]. Subsequently, the purified, DOTA-huJ591 was labeled with ^{90}Y using ammonium acetate buffer to produce specific activities of 200 MBq/mg (5.4 mCi/mg). The irrelevant MAbs, IA-1 and IA-2 were labeled with either ^{131}I or ^{90}Y using similar procedures described for huJ591.

LNCaP Tumor Model

Prostate carcinoma cell line LNCaP was grown in RPMI 1640, supplemented with 10% fetal calf serum, at a temperature of 37°C in an environment containing 5% CO_2. Prior to use, the cells were trypsinized, counted, and suspended in Matrigel (Collaborative Biomedical Products, Bedford, MA). Nu/Nu Balb C mice 8–10 weeks of age were inoculated, in the right and left flanks, with a suspension of 5×10^6 LNCaP cells in Matrigel (BD Biosciences, Bedford MA). After a period of 10–14 days, PSMA positive tumors (100–400 mg) had developed. The mice were divided randomly into several groups (n = 7–12 per group) and RIT and biodistribution studies were performed. All animal experiments were conducted in accordance with the Guidelines for the Care and Use of Research Animals established by Institutional Animal Care and Use Committee of Weill Medical College of Cornell University and complied with Federal and New York State regulations.

Control Studies and Tumor Growth

Prior to performing the RIT studies, control studies were first performed to assess tumor growth as a function of time and radioactivity. Three groups of mice bearing tumors 300–400 mg were injected, via the tail vein, with 0.2 ml of PBS containing, either ^{131}I-IA-1 (3.7 MBq) or ^{90}Y-DOTA-IA-2 (2.22 MBq) of irrelevant MAb. The third group received no injection (untreated control group). The animals were observed for periods of up to 8–10 weeks. At 3–4 day intervals the animals were weighed and the tumor size was measured bidimensionally [28] with a vernier caliper along the longest axis (x) and the axis perpendicular to the longest axis (y). The tumor volume was then estimated by using the following formula:

$$\text{Volume} = 4/3 \times \pi \times (x/2) \times (y/2)^2$$

The animal body weight was then adjusted for the tumor volume. The animals were humanely sacrificed if the tumor free body weight dropped below 80% of the starting mass or if the tumor mass exceeded 10% of the tumor free body weight.

RIT Studies

The anti-tumor effects of radiolabeled huJ591 were assessed in three separate studies. The first study was performed in mice bearing 300–400 mg tumors while studies 2 and 3 were performed in mice with 100–200 mg tumors. In the first study, two groups of mice received a single dose of ^{131}I-huJ591 (3.7 or 11.1 MBq) while a third group received a single dose of 1.3 MBq of ^{90}Y-DOTA-J591. In the second study three groups of mice received a single injection of 3.7, 5.55, or 7.4 MBq of ^{90}Y-DOTA-huJ591. In the third study, we evaluated the anti-tumor effect of multiple administrations of ^{90}Y-DOTA-huJ591. Three groups of mice received 1.11, 2.22, or 3.33 MBq. Subsequently, the mice in each group received two additional treatments at the same dose level at days 28 and 56 following the first treatment dose. The mice were checked daily for signs of toxicity and survival was monitored daily; the mouse weight and tumor size was measured at 3–4 day intervals for 2–6 months or until the death of animals by natural causes or sacrifice due to decrease in body mass greater than 20% of baseline value.

Initial tumor volume measured 1 day before treatment with radiolabeled antibody was regarded as the "baseline" value. Following treatment, tumor mass was normalized to the baseline value and expressed as a percentage of baseline value. For each group of mice, the median tumor volume was plotted against time (days) post treatment. If an animal died in any group, it was assigned the size rank it had at the last

measurement. A period of "tumor regression" was calculated to categorize the anti-tumor responses. The Kaplan–Meier plots of percentage survival as a function of time were generated to assess the toxicity and effect of treatment (or no treatment) on the survival of mice in each group. An estimate of the radiolabeled antibody dose lethal to 50% of mice is regarded as LD_{50}. The maximum tolerated dose (MTD) was defined as the highest dose that allows 100% of animals to survive longer than the survival of mice in control group (>MST) with less than 20% loss in men body weight.

Biodistribution and Radiation Dosimetry

In order to estimate the radiation dose delivered to the tumor and normal organs, biodistribution studies were performed with radiolabeled huJ591 in mice bearing LNCaP tumors. Mice were injected, via the tail vein, with 80–400 KBq (2.2–10.8 μCi) of the ^{131}I-huJ591 or ^{111}In-DOTA-huJ591. Groups of animals (3–8 per group) were sacrificed after 2, 4, or 6 days. The major organs and tumors were recovered. The tissue samples were weighed and counted, with appropriate standards in an automatic NaI(Tl) counter. These measured relative activity data (cpm) were background corrected and expressed as a percentage of the injected dose per gram (% I.D./g). These data were also fitted with a least squares regression analysis (Microcal Origin, Northampton, MA) to determine the rate of clearance of radioactivity from blood, tumor, and several organs.

Radiation dose to the tumor and normal organs was calculated based on biodistribution data and time-activity curves. Briefly, the radiation dose (cGy/MBq or rads/μCi) to any organ is a product of residence time, τ (μCi-hr) and S value (rads/μCi-hr) of the radionuclide for each of the source-target organ pair [29]. The τ values in various organs for ^{131}I-huJ591 were calculated based on biodistribution data. However, the τ values in various organs for ^{90}Y-DOTA-huJ591 were based on ^{111}In-DOTA-huJ591 biodistribution data. It has been well documented that ^{111}In behaves as a chemical and biological surrogate of ^{90}Y and that radiation dosimetry for ^{90}Y labeled tracers can be estimated based on the corresponding ^{111}In labeled tracers [30,31].

In the traditional models, often called macrodosimetric methods, β-particles and electrons from radionuclides are regarded as non-penetrating radiation. That is, the absorbed fraction (ϕ) is assumed to be unity for the source organ and zero elsewhere. But in a small animal such as mouse, the high energy β-particles, especially from ^{90}Y should be treated as penetrating radiation [32]. Therefore, we have used the corrected S values for ^{90}Y and ^{131}I, which do not assume 100% absorption of β-particles in the source organ [32]. Since absorbed dose to the tumor is dependent upon tumor size, we have estimated tumor doses for a mean tumor size of about 0.5 g. For the blood, the absorbed dose was calculated for a total blood volume of a mouse (1.5 ml) [33]. The dose to bone marrow was calculated using a marrow-to-blood activity concentration of 0.36 [33–35].

RESULTS

Control Studies With Nude Mice Bearing LNCaP Tumors

The first control group of mice received no treatment. Two additional control groups received an irrelevant MAb, ^{131}I-IA-1 (3.7 MBq) or ^{90}Y-IA-2 (2.22 MBq). Over the next 6–8 weeks, there was uncontrolled tumor growth (200–300%) in all three control groups (Fig. 1a). Tumor growth did not differ among the three control

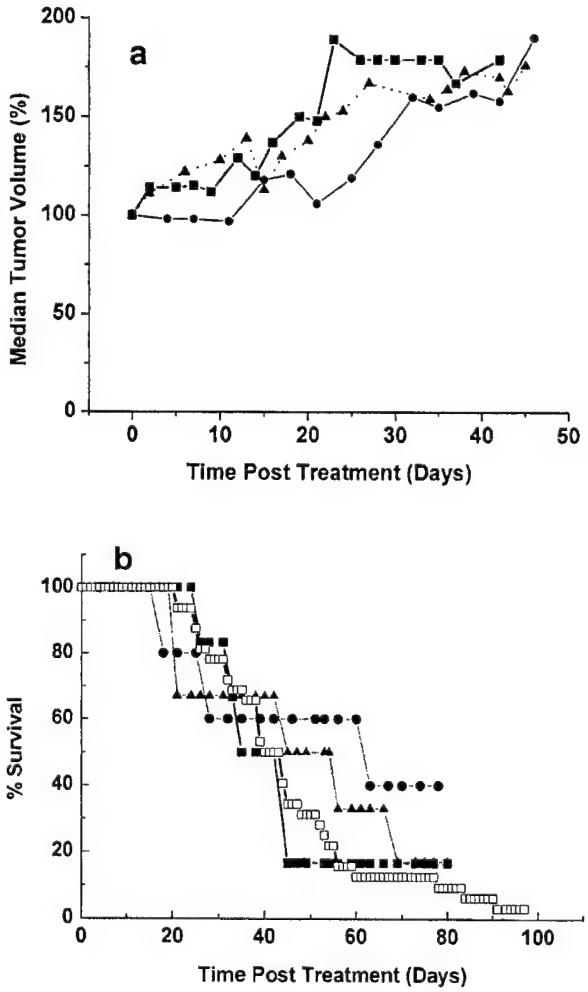

Fig. 1. Radiolabeled antibody treatment of nude mice bearing LNCaP xenografts: Control studies. (**a**): Effect on tumor growth, (**b**) effect on survival. ■ = untreated, no injection; ● = ^{131}I-irrelevant antibody-1 (3.7 MBq); ▲ = ^{90}Y-irrelevent antibody-2 (2.22 MBq); □ = average of all three controls.

groups. The tumor growth in untreated controls also did not differ from that of mice receiving radiolabeled control Mab. All mice experienced a loss in body mass and died naturally or were sacrificed. The Kaplan–Meier survival plots demonstrated no significant differences among the three control groups (Fig. 1b). The median survival time (MST) of tumor bearing mice in the control groups was 40 days.

RIT Studies With ^{131}I-huJ591

Two groups of tumor-bearing mice received a single dose of ^{131}I-huJ591 (3.7 or 11.1 MBq). The mice in 3.7 MBq group showed superior survival compared with controls (Fig. 2b). Among the two groups, the mean (or median) tumor reductions differed dramatically (Fig. 2a). No significant reductions were seen in the 3.7 MBq group (n = 10), even though a subset of six mice showed some evidence of tumor response. But substantial reductions occurred in the 11.1 MBq group (75% reduction over 35–40 days) (Fig. 2a). The 11.1 MBq dose was highly toxic, however, as 40% of mice died approximately 10 days after beginning treatment. With a single dose of ^{131}I-J591 (3.7 MBq), 80% of mice survived 56 days, and 20% survived 81 days. The MST for this group is at least 56 days.

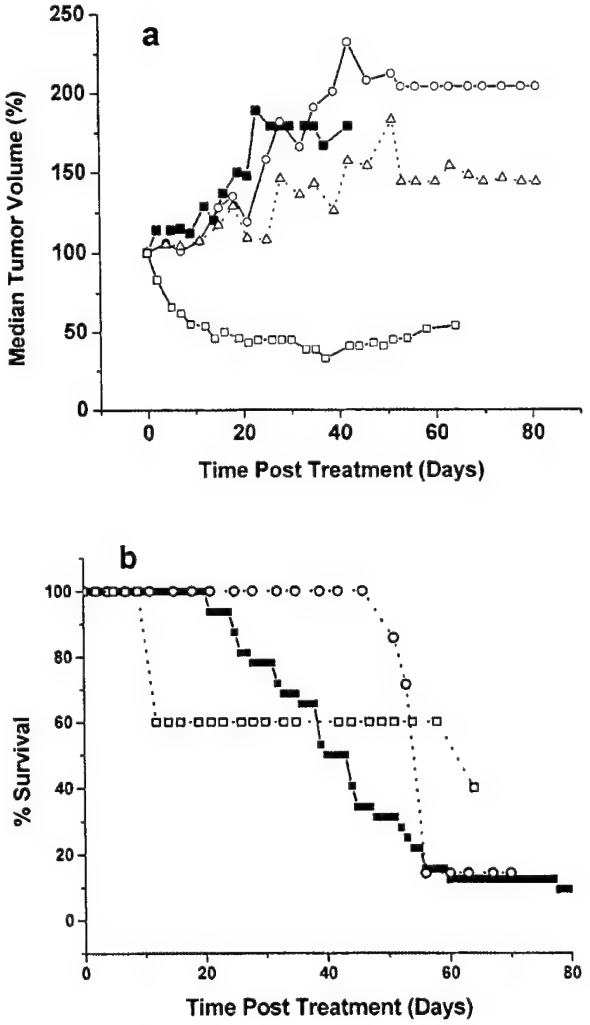

Fig. 2. ^{131}I-huJ591 antibody treatment of nude mice bearing LNCaP xenografts. (**a**): Effect of dose on tumor growth, (**b**) effect of dose on survival. ○ = 3.7 MBq (n = 10); △ = 3.7 MBq (n = 6, a subset); □ = 11.1 MBq; ■ = untreated, no injection.

RIT Studies With ^{90}Y-DOTA-huJ591: Single Dose

Four groups of mice received a single dose of 1.3, 3.7, 5.55, or 7.4 MBq of ^{90}Y-DOTA-J591. The group that received 1.3 MBq had 300–400 mg tumors while the other three groups had 100–200 mg tumors. In mice with smaller tumors, a clear anti-tumor (Fig. 3a) and survival (Fig. 3b) dose–response relationship was observed. Reduction in mean tumor volume at the 3.7, 5.55, and 7.4 MBq dose levels was 30, 55, and 90%, respectively. At all dose levels, tumor re-growth occurred. At 5.55 and 7.4 MBq, the delay in tumor re-growth was 35 and 60 days. With ^{90}Y-DOTA-J591, the MTD dose was 5.55 MBq and MST was 80 days. Mice with large tumors showed a 15% reduction and relative stabilization in tumor volume even at 1.3 MBq dose level. But the MST for this group is approximately 50 days.

RIT Studies With ^{90}Y-DOTA-huJ591: Multiple Doses

Three groups of mice received 1.11, 2.22, or 3.33 MBq of ^{90}Y-DOTA-huJ591 every 28 days for three doses (Fig. 4a,b). As with the single-dose studies, tumor reductions and survival appeared to be dose-dependent, but severe toxicity occurred at the highest dose (3.33 MBq). At day 60, there was a 50–70% reduction in the mean tumor size at the 2.22 and 3.33 MBq dose levels (minimal effect at 1.11 MBq). Compared to mice treated with a single dose of 5.55 MBq, the MST was longer (120 vs. 80 days) in mice treated with 1.11 or 2.22 MBq. Repeat injections of 3.33 MBq, however, resulted in greater toxicity and deaths after the 3rd dose with a MST of 60 days.

RIT and Body Mass

Treatment effects on body mass (Fig. 5) fell into three categories: (1) persistent loss of body mass (as in controls), (2) initial loss of body mass, followed by temporary body mass gains and subsequent body mass declines as tumor re-growth occurred (11.1 MBq) dose of ^{131}I-huJ591 and 5.55 MBq of ^{90}Y-DOTA-huJ591, and (3) stable body mass with for a substantial

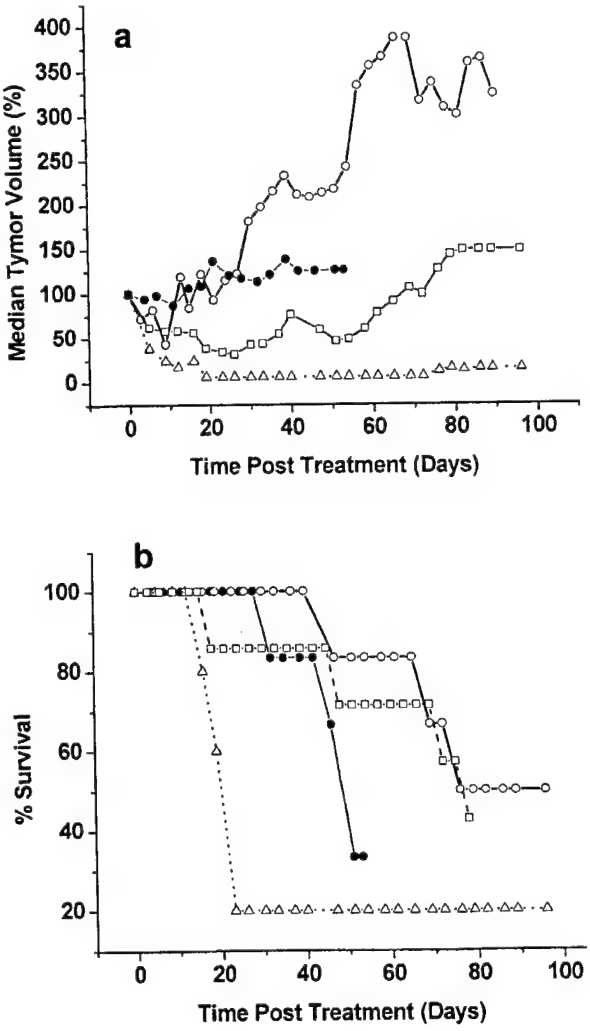

Fig. 3. ^{90}Y-huJ591 antibody treatment (a single dose) of nude mice bearing LNCaP xenografts. (a): Effect of dose on tumor growth, (b) effect of dose on survival. ● = 1.3 MBq; ○ = 3.7 MBq; □ = 5.55 MBq; △ = 7.4 MBq. Mice with relatively large tumors (300 – 400 mg) were treated with 1.3 MBq of ^{90}Y while the higher doses were studied in mice with 100 – 200 mg tumors.

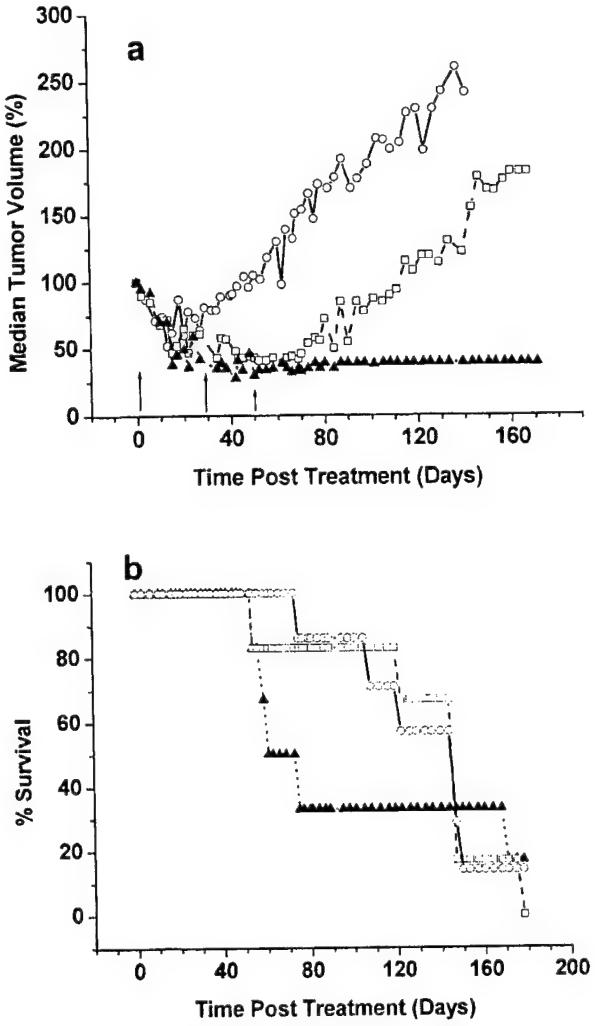

Fig. 4. ^{90}Y-huJ591 antibody treatment (multiple doses) of nude mice bearing LNCaP xenografts. (a): Effect of dose on tumor growth, (b) effect of dose on survival. ○ = 1.11 MBq; □ = 2.22 MBq; △ = 3.33 MBq.

period (2.22 MBq of ^{90}Y-DOTA-huJ591). Post injection body weight reductions typically occurred within 20–25 days and ranged from 10 to 15% with ^{131}I-huJ591 (11.1 MBq dose) and ^{90}Y-DOTA-huJ591 (5.55 MBq dose). The latter group regained to 95% of the initial body weight at day 40. In both groups, gradual gains in body mass typically occurred in parallel with a reduction in tumor volume. However, as the tumors started to re-grow, the animals again began losing body weight. In contrast, repeat administrations of 2.22 MBq of ^{90}Y-DOTA-huJ591 showed a markedly different pattern—significant decrease in tumor volume and retained a good body mass (100 ± 5%) over the next 12–14 weeks.

Biodistribution and Radiation Dosimetry of ^{131}I-J591 and ^{111}In-DOTA-huJ591

Localization of ^{131}I-J591 and ^{111}In-DOTA-huJ591 in selected organs and tumor tissue of nude mice bearing LNCaP tumors is shown in Table I. Since ^{111}In behaves as a chemical and biological surrogate of ^{90}Y, radiation dosimetry of ^{90}Y-DOTA-huJ591 was estimated based on ^{111}In-DOTA-huJ591 biodistribution data [30,31]. Based on mono-exponential clearance of blood time-activity curves, the rate of blood clearance of ^{131}I-huJ591 is slower than the ^{111}In-DOTA-huJ591 ($T_{1/2}$ = 4.2 vs. 2.3 days). By contrast, the tumor uptake of ^{111}In-DOTA-huJ591 was significantly greater than the uptake of ^{131}I-huJ591 (17.4 vs. 9.6% I.D./g on day 6). Similarly, the liver, kidney, and spleen uptake of

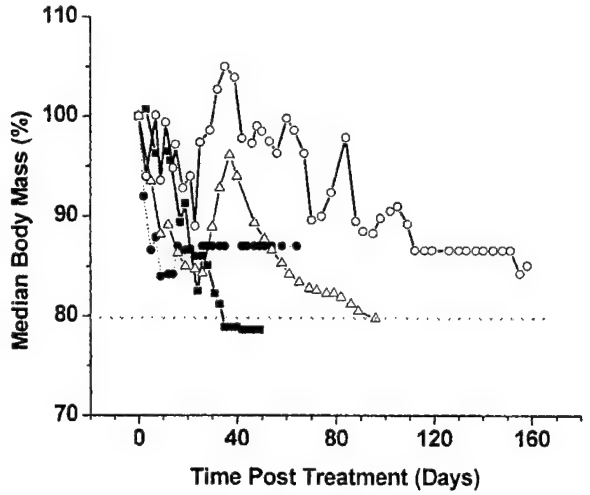

Fig. 5. Effect of radiolabeled antibody treatment on the body mass of nude mice bearing LNCaP xenografts. ■ = untreated, no injection; ● = ^{131}I-huJ591 (11.1 MBq); △ = ^{90}Y-huJ591 (5.55 MBq); ○ = ^{90}Y-J591 (2.22 MBq × 3 doses).

^{111}In-DOTA-huJ591 was also significantly greater than the uptake of ^{131}I-huJ591.

Based on biodistribution data and time-activity curves, the average radiation absorbed dose (cGy/MBq) estimates for ^{131}I-huJ591 and ^{90}Y-DOTA-huJ591 in tumor, blood, and bone marrow were calculated (Table II). In general, radiation dose to blood and tumor was higher with ^{90}Y than with ^{131}I. With 11.1 MBq of ^{131}I-huJ591, there was a 75% reduction in mean tumor volume and the tumor received approximately 2,766 cGy. But with 7.4 MBq of ^{90}Y-DOTA-huJ591, there was a 90% reduction in mean tumor volume and the tumor received approximately 3,674 cGy. However, at these dose levels, the bone marrow absorbed dose with ^{90}Y was almost 50% higher than that of ^{131}I (879 vs. 565 cGy).

DISCUSSION

We have previously reported that ^{131}I and ^{111}In labeled huJ591 bind with strong affinity (K_d 1.86 nM) to viable LNCaP tumor cells in vitro [25]. The biodistribution studies of radiolabeled huJ591 in nude mice clearly demonstrated specific tumor uptake in PSMA positive tumors only [26]. At 4 days post injection, the tumor uptake (% I.D./g) of ^{131}I-J591 is almost 20 times higher in PSMA-positive LNCaP tumors (11.4 ± 1.49) than in PSMA-negative PC3 (0.66 ± 0.07) and DU145 (0.55 ± 0.03) tumor xenografts.

The results of the present study clearly demonstrate the anti-tumor effect of ^{131}I-huJ591 and ^{90}Y-DOTA-huJ591 in the LNCaP xenograft model and support our hypothesis that radiolabeled huJ591 is an appropriate agent for RIT studies in patients with prostate cancer.

Anti-Tumor Effect of Radiolabeled huJ591

The anti-tumor effect of radiolabeled huJ591 as measured by the reduction of tumor size in PSMA-positive tumors is dose-dependent. The MST, however, depends very much on the size of the tumor at the time of treatment, on the radionuclide (^{90}Y vs. ^{131}I) and the dose of radiolabeled antibody. Following administration of a single dose of ^{90}Y-DOTA-huJ591 (3.7–7.4 MBq), there was a 30–90% reduction in mean tumor volume. Similarly, with ^{131}I-huJ591 (3.7–11.1 MBq), there was a 15–75% reduction in tumor volume. Multiple administrations of fractionated small doses of ^{90}Y-DOTA-huJ591 (total cumulative doses of 6.7–10 MBq also had a 50–70% reduction in the mean tumor size. The MST of mice with large tumors in the control groups was 40 days. In mice with similar size tumors, MST was increased to 56 days with a single dose of ^{131}I-huJ591 (3.7 MBq) and 50 days with 1.3 MBq of ^{90}Y-DOTA-huJ591. But in mice with smaller tumors, the MST was 80–100 days (at 3.7–5.55 MBq). With a

TABLE I. Biodistribution (% I.D./g) of ^{131}I-J591 and ^{111}In-DOTA-huJ591 in Nude Mice Bearing LNCaP Tumors

	Day-2		Day-4		Day-6	
Organ	^{131}I	^{111}In	^{131}I	^{111}In	^{131}I	^{111}In
Blood	8.57 ± 2.04[b]	8.98 ± 2.10[a]	5.96 ± 1.61[b]	4.78 ± 0.85[b]	4.42 ± 1.74[b]	2.52 ± 0.56[b]
Lung	4.65 ± 1.77	5.89 ± 0.30	3.35 ± 0.97	3.40 ± 0.32	2.29 ± 0.91	2.47 ± 0.65
Liver	2.71 ± 0.50	7.68 ± 0.50	2.06 ± 0.46	7.66 ± 2.44	1.31 ± 0.34	6.08 ± 0.83
Kidney	2.11 ± 0.57	5.25 ± 0.63	1.37 ± 0.24	5.39 ± 1.27	1.20 ± 0.51	4.53 ± 0.87
Spleen	2.88 ± 0.89	5.36 ± 1.25	2.33 ± 0.72	4.43 ± 0.89	1.74 ± 0.72	3.36 ± 0.61
Muscle	0.62 ± 0.19	0.67 ± 0.10	0.48 ± 0.24	0.55 ± 0.34	0.33 ± 0.18	0.30 ± 0.10
Tumor	11.2 ± 2.90	13.6 ± 0.28	11.4 ± 4.21	15.7 ± 3.50	9.58 ± 3.2	17.4 ± 3.50

Number of mice/group; [a] = 4 and [b] = 7 or 8.
Mean ± SD.

TABLE II. Radiation Absorbed Dose Estimates With ^{131}I-huJ591 and ^{90}Y-DOTA-huJ591

	^{131}I		^{90}Y	
Organ	cGy/MBq	cGy/11.1 MBq	cGy/MBq	cGy/7.4 MBq
Tumor	249	2,766	496	3,674
Blood	141	1,569	330	2,441
Bone marrow	51	565	119	879

Tumor size = 0.5 g, blood volume = 1.5 ml; BM/blood = 0.36.
cGy = rad; MBq = 27.02 μCi; 7.4 MBq = 200 μCi; 11.1 MBq = 300 μCi.

fractionated dose regimen, the MST was increased by almost 200%, to 120 days with ^{90}Y-DOTA-huJ591 (cumulative doses of 3.33 and 6.7 MBq).

The MTD for ^{90}Y-DOTA-huJ591 appears to be around 5.55 MBq. At a higher dose (7.4 MBq), 80% of the mice even with smaller tumors at baseline, died between 15–20 days post injection of radioactivity. Mice that received a single dose of ^{131}I-huJ591 (11.1 MBq) or ^{90}Y-DOTA-huJ591(5.55 MBq) showed a progressive loss in body weight (10–15%) with in 20–25 days but returned to 95% of the initial body weight. By contrast, mice treated with repeat administrations of low dose ^{90}Y-DOTA-huJ591 (2.22 MBq) retained a normal/baseline body mass (100 ± 5%) over the next 12–14 weeks.

Radiation Dosimetry

The absorbed radiation dose to the tumor with ^{90}Y-DOTA-huJ591 is twice compared to the dose with ^{131}I-huJ591 (496 vs. 241 cGy/MBq). Also, with ^{90}Y there is a direct linear relationship between tumor dose and the percentage decrease in tumor size (Fig. 6a) suggesting that below 1,000 cGy, there may not be any measurable anti-tumor response. However, a treatment with 0% tumor reduction may stop the tumor growth for a period of time (stabilization), which may also be regarded as a positive response. At the LD_{50} level (7.4 MBq), with a tumor dose of 3,674 cGy, there was a 90% decrease in tumor volume. In our study, the LD_{50} for ^{131}I-huJ591 is greater than 11.1 MBq. At this level with a tumor dose of 2,766 cGy, a 75% decrease in tumor volume is consistent with the observed dose–response relationship (Fig. 6a). Our dosimetry data is also consistent with previously published radiation dosimetry values for ^{90}Y and ^{131}I labeled MAbs. For several ^{90}Y-DOTA labeled MAbs at the MTD level, the absorbed doses to the tumor were between 1,698 and 4,882 cGy [36–40]. Similarly for ^{131}I labeled MAbs, the tumor doses were between 1,365 and 4,070 cGy [28,33,40,41]. This wide variation in tumor doses were due to differences in radiolabeling techniques, MAbs, nude mice tumor models, and radiation dosimetry methodology.

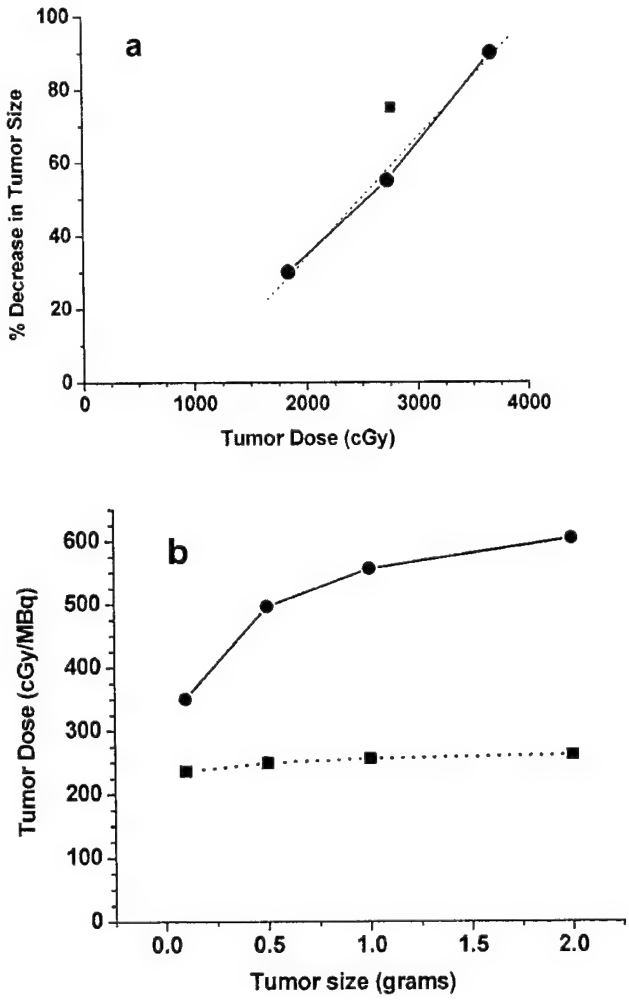

Fig. 6. Anti-tumor response and absorbed radiation dose following radiolabeled J591 antibody treatment in nude mice bearing LNCaP xenografts (**a**). Anti-tumor response (percentage decrease in tumor volume) as a function of absorbed radiation dose (cGy) to the tumor. 7.4 MBq of ^{90}Y-DOTA–huJ591 (tumor dose = 3,674 cGy) resulted in 90% reduction of tumor volume while 11.1 MBq of ^{131}I-J591 (tumor dose = 2,766 cGy) resulted in 75% reduction. (**b**) Absorbed radiation dose (cGy/MBq) to the tumor as a function of tumor size. Tumor dose (cGy) per tumor size (g) is much higher for ^{90}Ycompared to that with ^{131}I. ● = ^{90}Y-J591; ■ = ^{131}I-J591.

Due to its distinct radiosensitivity, the red marrow is the first-line dose-limiting organ in RIT [40]. Myelotoxicity (thrombocytopenia and leukopenia) between 1 and 3 weeks following administration of radiolabeled MAbs has been reported in mice [37,38,40]. The MTD for radiolabeled MAbs is dependent mostly on the extent of myelotoxicity. The radiation absorbed dose (cGy/MBq) to bone marrow was estimated to be between 119 and 148 for ^{90}Y labeled MAbs and 51–70 for ^{131}I labeled Mabs [28,33,36–41]. At MTD, the reported bone marrow doses were 547–889 or even as high as 1,200 cGy/MBq. One of the major factors for this wide range of values is that bone marrow doses are generally estimated based on the blood dose and bone marrow/blood ratios are generally assumed to be between 0.2 and 0.4 [40]. In tumor bearing mice, MTD for ^{90}Y labeled MAbs using bifunctional chelate DTPA was around 2 MBq and was partly due to the poor in vivo stability of the radiometal–chelate complex and greater bone uptake of ^{90}Y [42,43]. Subsequently, using DOTA analogs with greater in vivo stability of radiometal–chelate complex, the MTD for most of the ^{90}Y labeled MAbs was reported to be between 3 and 7 and 5.55 MBq [38–40] and even as high as 9.6 MBq [37].

In our studies, the dose to bone marrow with ^{90}Y-huJ591 is twice that of ^{131}I-huJ591 (119 vs. 51 cGy/MBq). Also the toxicity with ^{90}Y is greater compared to that with ^{131}I (Figs. 2b, 3b, and 6). The bone marrow dose with ^{90}Y at MTD (5.55 MBq) was 660 cGy and at LD_{50} (7.4 MBq) was 879 cGy. By contrast, with three injections of 3.33 MBq of ^{90}Y-DOTA-huJ591 (total 10 MBq), the cumulative bone marrow dose of 1,190 cGy was well tolerated by the mice with minimal toxicity. These observations clearly suggest that both total dose and dose rate are equally important for bone marrow toxicity [40] and that fractionated dose regimen with multiple administrations of smaller doses of radiolabeled MAb may be more advantageous and less toxic compared to a single high dose RIT treatment.

Choice of Radionuclide

Among the many radionuclides that are potentially useful for RIT, the β-emitters, ^{131}I and ^{90}Y have emerged as the primary choices for a number of reasons. Each of these two nuclides, however, has potential advantages and disadvantages. In vivo, radioiodinated MAb is dehalogenated and the free radioiodide is washed out of tissues, including the tumor tissue, and excreted in the urine (>60% within 48 hr). In contrast, the macrocyclic bifunctional chelating agent DOTA, when conjugated to MAb, binds ^{111}In and ^{90}Y with very high affinity and the complex is relatively stable in vivo. Following tumor localization, the radiometal is trapped within the cell, leading to higher accretion of radionuclide by the tumor. The other important difference between these two nuclides is the energy of the β-particle. ^{90}Y is a high energy isotope (E_{max} = 2.27 MeV) with a longer range in tissue (12 mm) and ^{131}I is a moderate energy isotope (Emax = 0.6 MeV) with relatively shorter range in tissue (2 mm). It has been suggested that ^{90}Y may be appropriate for larger tumors while ^{131}I may be more cytotoxic for smaller, micrometastatic lesions. O'Donoghue, et al. [44] suggested that for targeted radionuclide therapy, there is an optimal tumor size for cure. For a cure probability of 0.9, the optimal tumor size was estimated to be 28–42 mm for ^{90}Y compared to 2.6–5.0 mm for ^{131}I. It has been shown that the fraction of electron energy absorbed in small tumors (0.1–2.0 g) for high energy β-particles of ^{90}Y is directly related to the tumor size [44,45]. For a 0.1 g tumor, only 40% of energy is absorbed while a 2 g tumor absorbs about 75% of energy [46]. In the LNCaP tumor model, the dose to the tumor with ^{90}Y-DOTA-huJ591 is a function of the tumor size (Fig. 6b). Typically, most of the tumors in this model are 0.1–0.5 g and the absorbed doses are 350–500 cGy/MBq. In contrast, with ^{131}I, the differences in absorbed doses are less significant. As a result, there will be a wide range of anti-tumor responses observed with ^{90}Y in the animal models. Similarly, in patients, with micrometastases and less than 3–5 g tumors, there may be significant differences in the tumoricidal response with ^{90}Y labeled MAbs. It has also been suggested that nonuniform absorbed dose distributions within the tumor tissue due to heterogenous uptake of the radiolabeled MAb may lead to inefficient sterilization of the tumor cells [32]. The higher energy β-particles of ^{90}Y may therefore contribute "crossfire" radiation to tumor regions of low uptake. All the theoretical and practical considerations strongly suggest that ^{90}Y-huJ591 may be more appropriate than ^{131}I-huJ591 for RIT studies in patients with prostate cancer.

CONCLUSIONS

The results of the present study clearly demonstrate the anti-tumor effect of ^{131}I-huJ591 and ^{90}Y-DOTA-huJ591 in the LNCaP xenograft model and support our hypothesis that radiolabeled huJ591 is an appropriate agent for RIT studies in patients with prostate cancer. The anti-tumor effect of radiolabeled huJ591 in PSMA-positive tumors is dose-dependent. A 15–90% reduction in mean tumor volume was observed after a single dose of ^{131}I-huJ591 (3.7–11.1 MBq) or ^{90}Y-DOTA-huJ591 (3.7–7.4 MBq). The median survival time increased 2–3 times relative to untreated controls. Multiple administrations of fractionated doses of ^{90}Y-DOTA-huJ591 were even more effective with minimal toxicity. The MTD is 5.55 MBq for ^{90}Y-DOTA-huJ591

and 11.1 MBq for ^{131}I-huJ591. Both total dose and dose rate are equally important for bone marrow toxicity and that fractionated dose regimen with multiple administrations of smaller doses of radiolabeled MAb may be more advantageous and less toxic compared to a single high dose RIT treatment. Compared to ^{131}I, the higher energy β-particles of ^{90}Y may contribute "cross-fire" radiation to tumor regions of low uptake. All the theoretical and practical considerations strongly suggest that ^{90}Y-DOTA-huJ591 may be a suitable radiopharmaceutical for the treatment of prostate cancer.

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Targeted Systemic Therapy of Prostate Cancer With a Monoclonal Antibody to Prostate-Specific Membrane Antigen

Neil H. Bander, David M. Nanus, Matthew I. Milowsky, Lale Kostakoglu, Shankar Vallabahajosula, and Stanley J. Goldsmith

For the last 60 years, hormonal therapy has been the cornerstone of treatment of metastatic prostate cancer. Unfortunately, hormonal therapy is purely palliative and improved systemic therapies are necessary. Monoclonal antibodies (mAbs) have proven valuable in the treatment of several diseases including cancer. mAbs act by focusing an immune response on or by targeting delivery of highly cytotoxic agents to the cancer cells without targeting normal cells. Prostate-specific membrane antigen (PSMA) has been identified as an ideal antigenic target in prostate cancer. PSMA is the most well-established, highly restricted prostate cancer cell surface antigen. It is expressed at high density on the cell membrane of all prostate cancers, and after antibody binding, the PSMA-antibody complex is rapidly internalized along with any payload carried by the antibody. J591 is the first IgG mAb developed to target the extracellular domain of PSMA, and it has been deimmunized (humanized) to allow repeated dosing in patients. Three phase I studies are in progress, two using the β-emitting radiometals yttrium 90 and lutetium 177, and a third using a cytotoxin (DM1) linked to J591. Imaging of patients after they have received radiolabeled J591 demonstrates excellent tumor targeting.

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OVER THE LAST several years, monoclonal antibodies (mAbs) have repeatedly made the successful transition from the bench to the bedside with approximately a dozen mAbs now approved by the US Food and Drug Administration (FDA) for use in various clinical settings, including cancer therapy. mAbs have the benefits of being "natural" proteins that possess exquisite specificity and high affinity for their molecular target. In their native ("naked") form, mAbs possess the ability to initiate immunological effects, block receptors, or sequester ligands. Alternatively, they may be used as tumor-targeting mAb vehicles (T-MAVs) to deliver highly cytotoxic radionuclides, drugs, or toxins to the desired cell population. In the cancer field, antibodies have demonstrated therapeutic benefit in all of these formats: naked mAb for treatment of non-Hodgkins' lymphoma (NHL), in combination with conventional chemotherapy in breast cancer, as radiolabeled antibody in NHL, and as a cytotoxin-conjugate in acute myelogenous leukemia. The field of targeted cancer therapeutics was the subject of a recent excellent review.[1]

Prostate cancer represents an excellent target for mAb-based therapies for many reasons: (1) the prostate is a nonessential organ, thereby allowing targeting of organ- or tissue-specific antigens rather than requiring the identification of the more elusive cancer-specific antigens; (2) prostate cancer metastases predominately involve the bone marrow and lymph nodes, locations that receive high levels of circulating antibody and have proven responsive to mAb therapies in other tumor types (eg, lymphoma, breast cancer); (3) mAbs can mediate antitumor effect by targeting radionuclides and prostate cancer is relatively radiosensitive; (4) prostate cancer metastases are typically of small volume, allowing for ready antibody penetration and antigen access; (5) the availability of a sensitive blood test such as serum prostate-specific antigen (PSA) provides an indication for mAb therapy at the first sign of relapse, years before clinical manifestations of disease, when tumor volume is small and ideally suited for antibody delivery; (6) clinically validated measures exist to predict, even before PSA failure, those patients at high risk, allowing initiation of therapy in the face of an extremely small tumor

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burden; and (7) a surrogate marker such as PSA allows rapid clinical evaluation of potential therapeutic efficacy in phase I and II trials.

PROSTATE-SPECIFIC MEMBRANE ANTIGEN

Prostate-specific membrane antigen (PSMA) is the single most well-established, highly restricted prostate epithelial cell membrane antigen known.[2-7] The gene has been cloned, sequenced,[3] and mapped to chromosome 11p.[8] Although first thought to be entirely prostate-specific,[2-4] subsequent studies demonstrated that PSMA is also expressed by cells of the small intestine, proximal renal tubules, and salivary glands.[6] However, the level of expression in these nonprostate tissues is 100- to 1,000-fold less than in prostate tissue,[7] and the sites of PSMA expression in these normal cells (brush border/luminal location) are not typically exposed to circulating antibodies. In contrast to other well-known prostate-restricted molecules such as PSA and prostatic acid phosphatase (PAP) that are secretory proteins, PSMA is a type II integral cell-surface membrane protein that is not secreted, thereby making PSMA an ideal target for mAb therapy. Pathology studies indicate that PSMA is expressed by virtually all prostate cancers.[8] Moreover, PSMA expression increases progressively in higher-grade cancers, metastatic disease, and hormone-refractory prostate cancer.[4,5,9,10]

PSMA has been found to have folate hydrolase and neurocarboxypeptidase activity.[11] Although its role in the biology of prostate cancer is unknown, the consistent finding of PSMA upregulation correlating with increased aggressiveness of the cancer implies that PSMA does have a functional role. Inhibition of enzymatic activity in vitro or in xenograft models has not demonstrated significant growth inhibitory effect (Bander et al, unpublished data). Nevertheless, the expression pattern of PSMA makes it an excellent target for mAb-based targeted therapy of prostate cancer.

Initial validation of PSMA as an in vivo target has been demonstrated by imaging trials with mAb 7E11/CYT-356,[12,13]marketed as capromab pendetide (ProstaScint, Cytogen Corp, Princeton, NJ). Capromab is FDA-approved for imaging soft tissue sites of prostate cancer, though not for targeting/imaging bone metastases, the most common site of spread. Molecular mapping revealed that mAb 7E11/CYT-356/capromab targets a portion of the PSMA molecule that is within the cell's interior and not exposed on the outer cell surface.[16-18] Studies have shown that, because its intracellular epitope is masked by the cell's plasma membrane, 7E11/CYT-356 cannot bind to viable cells.[18] Interestingly, this finding was apparent from the first description of the 7E11 antibody, where it was noted that 7E11 could bind only fixed, but not viable, LNCaP cells.[2] This characteristic of 7E11/CYT-356/capromab is also thought to explain the basis of its ability to target soft tissue sites but not bone metastases. In the former site, lesions may outgrow their blood supply, causing foci of cell death and plasma membrane disruption and thereby exposing the intracellular epitope for capromab binding. Conversely, in the bone marrow, the small foci of tumor are well vascularized and viable, without the critical cell death necessary to expose the intracellular epitope. Recognition of these features led us and others to propose that mAbs to the exposed, extracellular domain of PSMA had the potential to significantly improve in vivo targeting, likely resulting in enhanced imaging and therapeutic benefit.[15,18]

MONOCLONAL ANTIBODIES TO $PSMA_{ext}$

Following our hypothesis, we produced the first series of IgG mAbs to $PSMA_{ext}$ (J591, J415, J533, and E99).[18] Enzyme-linked immunosorbent assays (ELISAs) confirmed that these mAbs to $PSMA_{ext}$ bound to the same molecule as 7E11 but did not compete with 7E11 for binding.[18] Immunoprecipitation and Western blot assays confirmed unmistakably that these mAbs bound to the 100-kd PSMA.[18] In contrast to 7E11/CYT-356, these mAbs recognize two distinct epitopes located on the exterior of the cell.[18,19] Furthermore, these antibodies demonstrate high-affinity binding to viable LNCaP cells in tissue culture[18-21] (Figs 1 and 2) The J591, J533, and E99 epitopes have been mapped using truncated PSMA constructs to amino acid residues 153 to 347, whereas J415 binds near the C-terminus (Suzuki and Bander, unpublished data). Of the four mAbs, only J415 inhibits enzymatic activity, consistent with the proposed catalytic region of PSMA.[22] Scatchard analysis indicates that two PSMA-expressing cell lines (LNCaP and MDA-Pca2b) express 1 million or more sites per cell surface.[20,21] Furthermore, as these were the first mAbs to PSMA that could bind viable cells, we were able to make the unan-

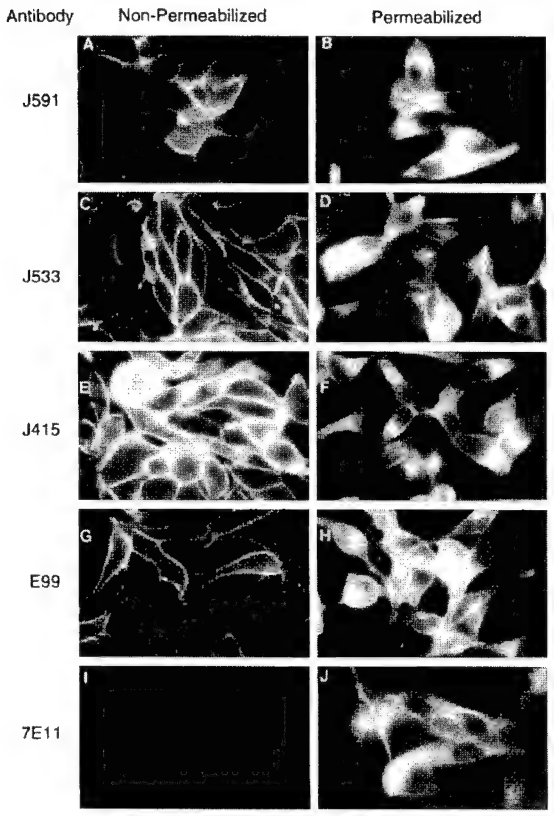

Fig 1. Immunofluorescence assay comparing the binding of anti-PSMA antibodies to nonpermeabilized and permeabilized PSMA-positive LNCaP cells. In nonpermeabilized cells, only antibodies binding to extracellular epitopes of PSMA (J591, J415, J533 and E99) bind (A, C, E, G). 7E11 (capromab), which recognizes an intracellular epitope of PSMA, cannot bind to intact, nonpermeabilized cells (I). When the cells are permeabilized prior to antibody incubation, binding to cytoplasmic as well as membrane PSMA is seen (B, D, F, H, J). (Reprinted with permission from *Cancer Research*.[18])

ticipated observation that, once bound, PSMA-antibody complexes are rapidly internalized[19] (Figs 3 and 4). This characteristic added to the appeal of PSMA, as it supported the feasibility of first targeting and then internalizing cytotoxins or isotopes conjugated to the mAb.

Murine monoclonal Ab J591 (muJ591) was chosen for clinical development and has been extensively studied in preclinical models.[20,21,23] The affinity of J591 is 1 nm, which other studies have shown to be the optimal affinity in therapeutic models, lower affinity providing less binding and higher affinity interfering with antibody penetration into tumor masses.[24]

Immunohistochemistry studies with mAbs to $PSMA_{ext}$ confirmed the highly restricted expression pattern of PSMA with binding to prostate epithelial cells and weak binding to the brush border of renal proximal tubular and small bowel epithelium. Unexpectedly, when we performed immunohistochemical studies of a variety of malignant tissues, we found that tumor vascular endothelium of all solid tumors, but not normal vascular endothelium, bound anti-PSMA antibodies.[18] This further raised the interest in anti-PSMA antibodies as a potential way to specifically target not just prostate cancer but all solid tumors using a vascular targeting approach.

A major limitation of using a mouse mAb in patients is the development of a human anti-mouse antibody (HAMA) response that precludes repetitive dosing. Therefore, mAb J591 was deimmunized by using a next generation approach to humanization developed by Biovation, Ltd (Aberdeen, UK). This technology involved sequencing of the J591 F(ab) regions followed by computer analysis of the sequence to identify mouse immunoglobulin sequence motifs recognizable by human B and/or T cells.[25] Potentially antigenic mouse sequences were replaced by human homologous sequences that would be nonimmunogenic

Fig 2. Immunoelectron photomicrographs demonstrating binding of (A) J591 and (B) 7E11 to viable LNCaP cells at 4°C. Antibody binding is indicated by the radiodense immunogold beads. (A) In the case of J591, binding can be seen on the extracellular aspect of the cell membrane. (B) No binding of 7E11 (capromab) is seen. N, nucleus. (Reprinted with permission.[18]) Troyer et al have done immunoelectron microscopy using LNCaP cells after fixation in which 7E11 binding is seen at the intracellular aspect of the plasma membrane.[19]

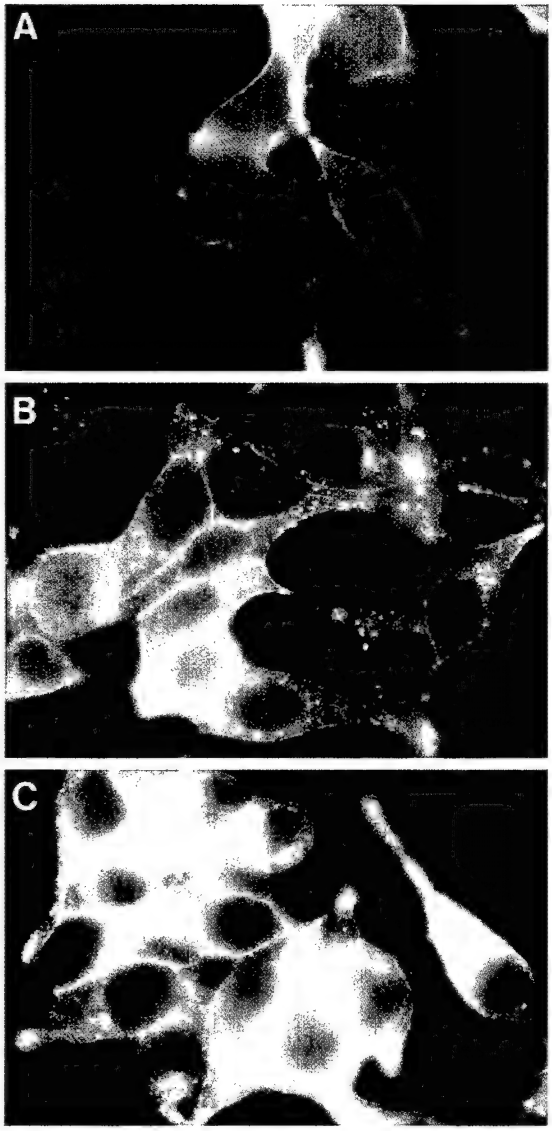

Fig 3. Immunoflorescence photomicrographs demonstrating internalization of J591 by viable LNCaP cells over time. Cells were incubated with J591 at 37°C for (A) 5, (B) 20, and (C) 180 minutes. Cells were then permeabilized and stained with secondary fluorescein-labeled antibody to visualize internalized J591. (Reprinted with permission.[19])

in patients. Candidate constructs were assayed for specificity and affinity and their sequences reanalyzed to confirm that no immunogenic motifs were created. The process is repeated until one selects the construct sequence with the optimal specificity and affinity and least anticipated immunogenicity. To the deimmunized F(ab) regions, a human IgG1 Fc region was used in order to add the potential for inducing antibody-dependent cellular cytotoxicity (ADCC) with human immune effector cells.

Effector Options

Effector options for J591 include the naked antibody's ability to mediate ADCC or the antibody's ability to deliver and internalize a payload of a therapeutic radionuclide or cytotoxin. The naked antibody approach using ADCC would probably be most effective in a clinical setting of limited disease burden such as adjuvant or neoadjuvant use in high-risk patients or early PSA failures. However, given the ability of J591 to specifically target and internalize a highly cytotoxic payload, it seems reasonable to take advantage of these properties in patients with more advanced disease where there is the most pressing need for improved therapy and where registration trials are likely to need to focus first. The ability to target and internalize offers significant advantages: the therapy is directed only to tumor cells thereby minimizing toxicity, an important factor in this often elderly population, and the ability, because of the specific targeting, to use cytotoxins substantially more potent than conventional, untargeted chemotherapy agents.

In the radionuclide area, the therapeutic beta-emitters iodine 131, yttrium 90, and lutetium 177 are appropriate candidates. There is already substantial experience with ^{131}I- and ^{90}Y-labeled mAbs, with ^{177}Lu having more modest experience.

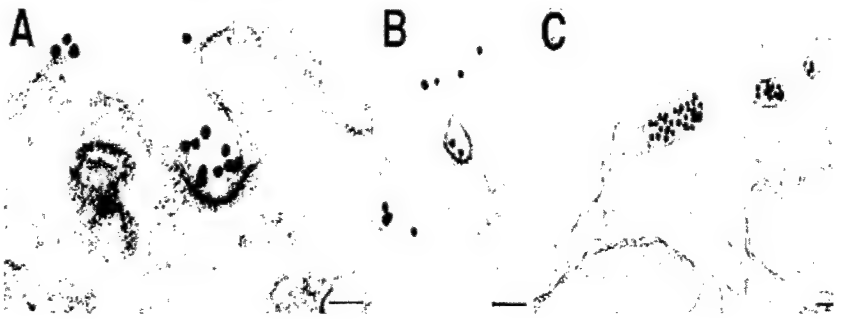

Fig 4. Immunoelectron micrograph of internalized J591 in LNCaP cells. Viable cells were incubated with J591 at 37°C for 10 minutes and then processed for immunogold labeling. J591/gold particles can be seen in clathrin-coated pits (A, B) and in endocytosed vesicles subjacent to the plasma membrane (C). Bars represent 34 (A) and 65 (B, C) nm, respectively. (Reprinted with permission.[19])

Table 1. Properties of Beta-Emitters Used in mAb-Targeted Therapies

	^{131}I	^{90}Y	^{177}Lu
Half-life (d)	8.05	2.67	6.7
β-emitter max (MeV)	0.61	2.28	0.497
β-emitter average (MeV)	0.20	0.935	0.149
γ (MeV)	0.364 (81%)	NONE	0.208 (11%)
			0.113 (7%)
Range (mm) max	2.4	12.0	2.20
Range (mm) average	0.4	2.7	0.25

The physical properties of these agents are provided in Table 1. Radioiodine can be directly linked to the antibody; however, internalization of an iodinated antibody results in rapid enzymatic dehalogenation of the internalized protein and rapid loss of the isotope by passive diffusion of the ion through the cell membrane. Radiometals, like ^{90}Y and ^{177}Lu, require a chelating agent to link the radionuclide to protein. The chelating agent is covalently linked to the protein and serves as a "molecular cage" to bind the radiometal. Once internalized, radiometals behave far differently from radioiodine in that the metals become irreversibly trapped within the cell.[21] ^{90}Y is a pure beta-emitter and cannot be imaged by external gamma cameras and, therefore, requires the use of indium 111 as a surrogate for imaging. Conversely, ^{177}Lu emits gamma radiation in addition to its beta particle. As a result, ^{177}Lu can be imaged directly, without need for a surrogate such as ^{111}In.

Our in vitro studies of J591 labeled with these three isotopes and their internalization by LNCaP cells confirmed expectations: ^{131}I had a relatively short intracellular half-life, whereas ^{90}Y and ^{177}Lu had intracellular half-lives more than 20-fold longer (>500 hours).[21] Clearly, the longer intracellular half-life is beneficial and important in order to deliver the maximal radiation to the target cell. Between the choices of radiometals, ^{90}Y had a shorter physical half-life and longer range than ^{177}Lu. The longer half-life and shorter range of ^{177}Lu offers benefits by allowing a longer time for the antibody-isotope to localize to tumor and the longer half-life also mates well with the long intracellular half-life. In addition, ^{177}Lu's shorter range would cause less bystander radiation to tissues neighboring tumor sites at the possible cost of less efficacy in bulkier lesions. The longer range of ^{90}Y offers benefits in being better able to radiate bulkier lesions. Beyond the theoretical advantages and disadvantages of the various isotopes, we chose to look at all three in a LNCaP xenograft model (see below).

Similarly, we are interested in exploring J591 as a T-MAV for a potent cytotoxin. We chose DM1, a derivative of maytansine. Maytansine is a cytotoxic tubulin-inhibiting compound originally isolated from the bark of an East African shrub, *Maytenus ovatus*. In the past, maytansine was evaluated for antitumor activity by the National Cancer Institute, but in an untargeted approach, it was associated with dose-limiting toxicities to the gastrointestinal and nervous systems[26] and was not developed further for clinical use. DM1 has undergone animal studies and continues in human studies linked to various antibodies in colon cancer and non-small cell lung cancer (NSCLC). DM1-antibody conjugates are not active unless and until the DM1 is internalized and released from antibody within the cell.

Animal (xenograft) Models

Animal studies used nude mice implanted subcutaneously with LNCaP cells. After allowing the tumors to establish and reach a diameter of 7 to 10 mm, the animals were treated with radiolabeled J591. Various control groups included animals receiving radiolabeled 7E11, naked J591, and radiolabeled irrelevant isotype-matched antibody. Tumor and organ dosimetry and pharmacokinetics were determined. The findings of these studies can be summarized as follows: (1) comparing 7E11 to J591, we found less difference in tumor dosimetry than expected based on the presumed better in vivo localization of the J591 antibody targeting the extracellular domain.[23] Autoradiography, however, provided the explanation. These tumors are typically highly necrotic. The autoradiograms demonstrated that 7E11 exclusively localized to necrotic areas of tumor whereas J591 preferentially localized to viable areas of tumor[23] (Fig 5). As the necrotic area generally made up at least half of the tumor, this explained the higher uptake of 7E11 relative to predictions. (2) Comparing ^{131}I to the radiometals confirmed the expectation that the radiometals would provide better dosimetry due to their longer intracellular half-life and radioiodine's

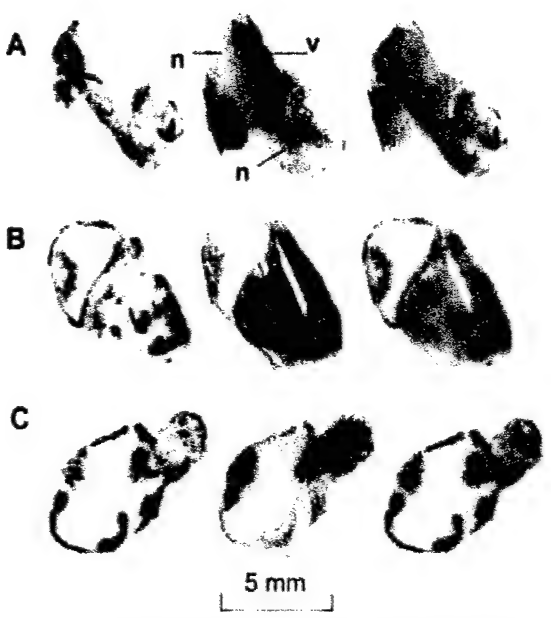

Fig 5. Autoradiographs and hematoxylin and eosin (H&E)-stained sections of LNCaP xenografts harvested 4 to 6 days after intravenous injection of $_{131}$I-labeled 7E11 (A), J591 (B), and J415 (C). Column 1, autoradiograph; column 2,= H&E section; column 3, composite of autoradiograph and H&E sections. Large areas of necrosis (n) were present in all tumors. Focality of mAb localization is evident in the autoradiographs. Also evident is the preferential uptake of 7E11 to areas of necrosis (n) and J591 and J415 to areas of viable (v) tumor. (Reprinted by permission of the Society of Nuclear Medicine from: Smith-Jones, PM, et al. Radiolabeled Monclonal Antibodies Specific to the Extracellular Domain of Prostate-Specific Membrane Antigen: Preclinical Studies in Nude Mice Bearing LNCaP Human Prostate Tumor. *J Nucl Med 2003;* 44(4):610-617.)

relatively rapid clearance from the tumor. (3) Antitumor responses were seen with all radionuclides with an apparent dose-response relationship. (4) The maximum tolerated dose (MTD) of ^{177}Lu-J591 was higher than that for ^{90}Y-J591. (5) Higher cumulative doses of either ^{90}Y or ^{177}Lu could be delivered using fractionated dosing (multiple sub-MTD doses rather than a single MTD dose) and this also yielded further improvements in response rates and survivals. Median survival improved by 300% for fractionated ^{90}Y-J591 therapy (150 days *v* 52 days [control]). With fractionated-dose ^{177}Lu-J591, more than 80% of the mice were cured (Smith-Jones P, et al, manuscript in preparation).

Clinical Trials in Prostate Cancer Using HuJ591.

Phase I trial of ^{111}In trace-labeled huJ591. The initial study using huJ591 was a phase I trial in patients with progressive prostate cancer.[27,28] The objectives of this trial were to define the toxicity,MTD, pharmacokinetics, biodistribution, and incidence of developing a human anti-humanized (deimmunized) antibody (HAHA) response to huJ591. Patients received four weekly doses of huJ591 trace-labeled with ^{111}In using a DOTA chelate. The initial dose levels (week 1) of huJ591 were 25, 50, 100, and 200 mg/m^2, with the maintenance doses (weeks 2, 3, and 4) at 50% of the initial dose. All patients received a single 4-week treatment course resulting in total doses of up to 500 mg/m^2. Therapy was well tolerated at all dose levels without toxicity, with the exception of one patient who experienced hypotension due to a rapid infusion rate. Subsequently, the infusion rate was limited to 5 mg/min and no further such reactions have occurred. No dose-limiting toxicity occurred and the MTD was not reached. Despite repeated dosing, no evidence of a HAHA response was detected in any patient.

After the first dose, total-body gamma camera images were obtained within 1 hour post-infusion (day 0) and on three more occasions in the following week. Excellent tumor targeting could be detected at all dose levels of mAb (Fig 6). No mAb targeting to non–prostate cancer sites was observed, although, as seen in other trials using radiometals, liver uptake is the primary site of excretion. Percent injected dose in the liver diminished with increasing dose of antibody, and higher doses were associated with longer plasma clearance times (S. Vallabajosula et al, manuscript in preparation).

Phase I trials of radiolabeled huJ591. Two independent phase I clinical trials were initiated using ^{90}Y or ^{177}Lu linked via a DOTA chelate to huJ591 in patients with hormone-refractory prostate cancer. The primary objectives of these trials were to define the MTDs of the isotopes, as well as to further define dosimetry, pharmoacokinetics, and HAHA of the radiolabeled mAb conjugates. Antitumor responses were assessed as a secondary end point. The design of and entry criteria for the two trials were identical. Eligible patients had a prior histologic diagnosis of prostate cancer. As prior studies had demonstrated that all prostate cancers were PSMA-positive,[8] no determination of PSMA expression was done and all hormone-refractory prostate cancer patients were eligible. Patients required evidence of progressing recurrent or meta-

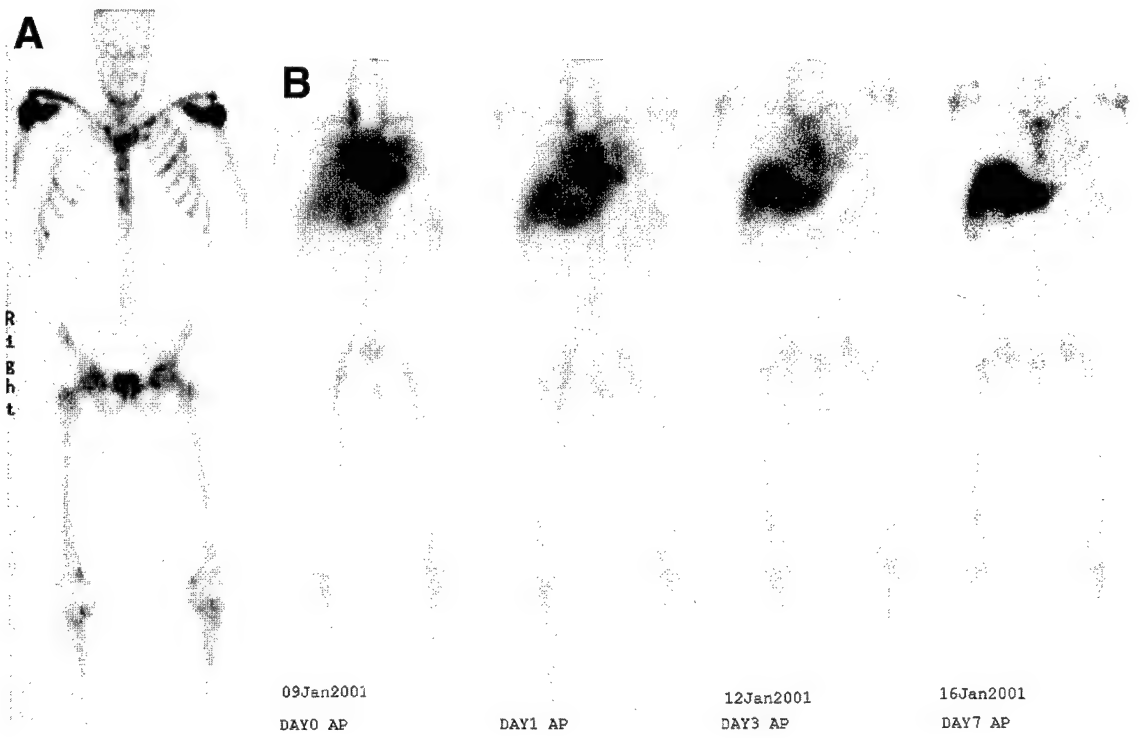

Fig 6. Anterior projection of (A) bone scan and (B) ^{111}In-J591 scan from the same patient. Bone scan demonstrates a "superscan" with cancer involving the entire skeleton and no excretion of nuclide via the urinary tract. The antibody scan (B) demonstrates that on day 0 (within 1 hour of injection), radiolabeled J591 can be seen predominately in the circulation (in the large vessels and heart). However, even at this early time point, localization of the radiolabeled J591 can already be seen in the tibias bilaterally. As time elapses, radiolabeled J591 leaves the circulation and localizes to the disease in the bone. Individual ribs can be seen, as can the bones of the extremities and pelvis. Excretion of the radiometal and nonspecific antibody clearance through the liver can be seen.

static disease defined by at least three serially rising PSAs and/or radiographic studies such as computed tomography (CT), magnetic resonance imaging (MRI), and/or bone scan demonstrating progression. Patients were required to have an absolute neutrophil count (ANC) $\geq 2.0 \times 10^9$/L, platelet count $\geq 150 \times 10^9$/L, and unilateral or bilateral posterior iliac crest bone marrow biopsy demonstrating $\leq$10% or $\leq$25% of the intratrabecular marrow space involved by prostate cancer, respectively. Patients were not permitted to receive corticosteroids, adrenal hormone inhibitors, or PC-SPES within 4 weeks of entry or chemotherapy and/or radiation therapy within 6 weeks of entry. Prior radiation therapy encompassing greater than 25% of the skeleton or prior treatment with strontium 89 (Metastron, Medi-Physics, Arlington Heights, IL) or samarium 153 (Quadramet, Berlex, Richmond, CA) were not permitted. Additional exclusion criteria included serum creatinine greater than 2.0 mg/dL, serum AST $\geq$ 2.0 times the upper limit of normal (ULN), serum total bilirubin $\geq$ 1.5 times the ULN, and serum calcium $\geq$ 12.5 mg/dL. Patients were required to have a normal coagulation profile (prothrombin time [PT] and partial thromboplastin time [PTT]) unless on anticoagulant therapy.

Patients were treated in the New York Presbyterian Hospital General Clinical Research Center(GCRC). In the ^{90}Y-J591 trial, patients initially received 5 mCi of ^{111}In linked via a DOTA chelate to 20 mg of mAb J591 for pharmacokinetic and biodistribution determinations. One week later, they received ^{90}Y–DOTA-J591. Gamma camera imaging for biodistribution and dosimetry was done during the week between the ^{111}In and ^{90}Y-J591 doses. All patients received 20 mg of mAb J591 with the ^{90}Y dose-escalated in cohorts of three or more patients at the following planned dose levels: 5, 10, 15, and 20 mCi/m^2. A fifth dose level of 17.5 mCi/m^2 was added to more precisely define the MTD. A 6- to 8-week observation pe-

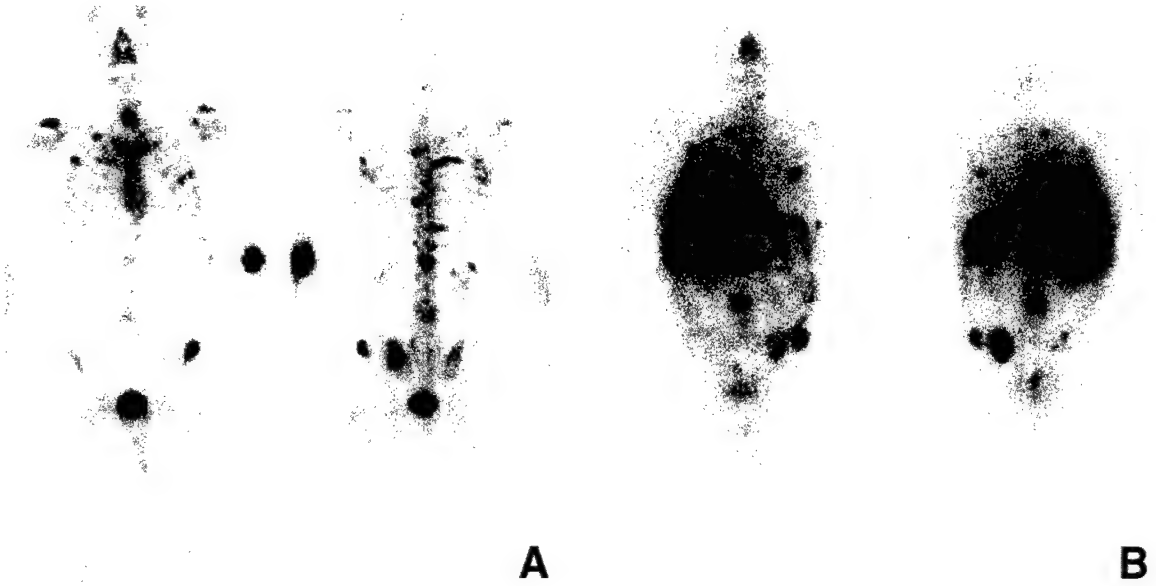

Fig 7. (A) Bone scan shows excretion through kidneys and bladder as well as multiple areas of increased uptake in ribs, spine, and pelvis. The injection site is apparent in the left antecubital fossa. (B) J591 scan, in addition to liver excretion of radiometal, shows areas of J591 targeting superimposable with the bone scan. The J591 scan shows more intense uptake than the bone scan, as well as some additional lesions (superior to dome of liver, L4, right SI joint, both proximal femurs) not seen in the bone scan.

riod between dose levels was required. All mAb administrations were by intravenous infusion. In the ^{177}Lu trial, patients received a total of 10 mg/m^2 of J591 with escalating doses of ^{177}Lu. Since ^{177}Lu can be directly imaged and has a longer physical half-life than ^{111}In, imaging took place during the 2 weeks following dosing. Accrual in the ^{90}Y trial has been completed at 29 patients; accrual in the ^{177}Lu trial continues at its seventh dose level of 75 mCi/m^2.

Dose-limiting toxicity in the two trials was defined as (1) hematologic toxicity consisting of grade 4 thrombocytopenia (platelets $< 10 \times 10^9$/L) and/or grade 4 neutropenia (ANC $< 0.5 \times 10^9$) for more than 5 days; and (2) other toxicity consisting of grade $\geq$ 3 nonhematologic toxicity attributable to radiolabeled J591.

Patients were monitored for a minimum of 12 weeks. Routine clinical and laboratory assessments (including biochemical profile, PSA, PAP, and testosterone) were performed at defined intervals. Compelte blood cell and platelet counts were initially monitored 1 to 2 times per week and then every 4 weeks until blood count stabilization. Chest x-ray, CT or MRI of the abdomen and pelvis, and a bone scan were performed post-treatment week 12. HAHA response was monitored at defined time points.

After the first 53 patients had been entered in the two trials, an analysis of J591 targeting was performed by comparing the J591 images to conventional imaging studies.[29] Of 53 patients in this series, 46 (87%) had radiographic evidence of metastatic disease while seven (13%) patients had no visible osseous or soft tissue lesions. Thirty-four patients (64%) had metastases demonstrated on radionuclide bone scans. Twenty-one patients (40%) had soft tissue lesions $\geq$ 2cm, defined as metastatic disease, demonstrated on CT. Three of these patients whose disease exclusively involved the liver were excluded from comparative evaluation with J591 imaging. Overall, of the 43 evaluable patients, J591 accurately targeted bone and/or soft tissue lesions in 42 (98%) patients[29] (Figs 7 and 8).

Of the seven patients with neither osseous nor soft tissue lesions visible on conventional imaging, three (43%) demonstrated J591 imaging of metastatic lesions either in the bone or soft tissue, all of which were confirmed by subsequent MRI or CT studies.[29]

The targeting accuracy of J591 scans was com-

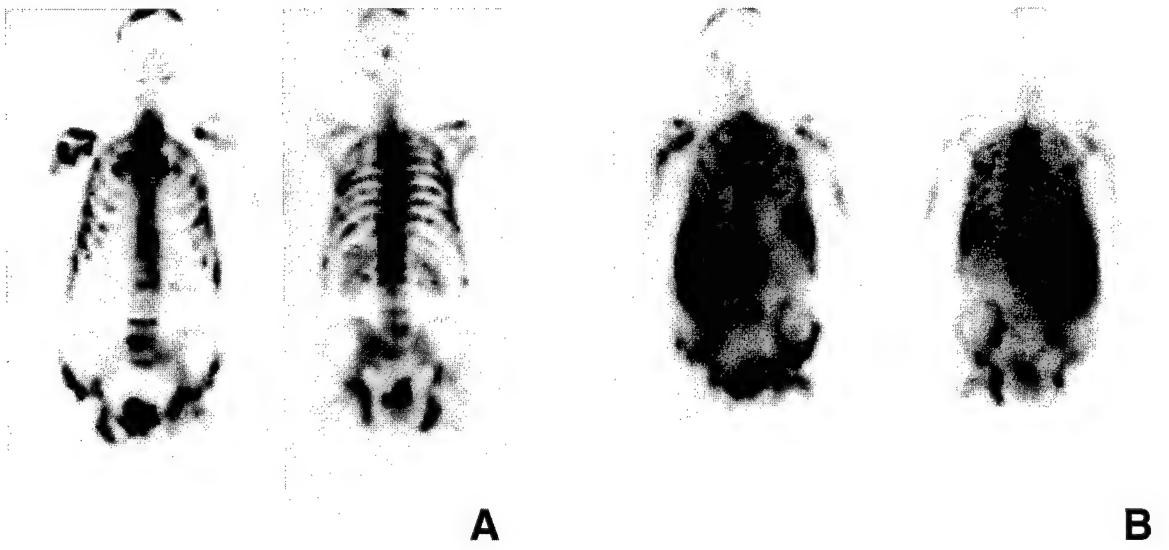

Fig 8. (A) Anterior and posterior bone scan and (B) J591 antibody scan showing localization of radiolabeled J591 to all sites of bone metastases. Metastatic sites are apparent in the cranium, upper extremities, ribs, spine, and pelvis. The patient had received prior radiation therapy to the lumbar spine, which showed no increased uptake on either bone scan or J591 scan. The J591 scan visualizes involvement of the marrow of the humeri bilaterally, not seen on the bone scan.

pared with conventional imaging, stratified by site(s) of disease: of the 34 patients with osseous lesions on bone scan, 32 (94%) demonstrated bone lesion targeting with J591. Of the 18 patients with no evidence of bone metastasis on conventional imaging, 16 (89%) had congruent results with J591 imaging. The two "false-positive" J591 scans were later confirmed to be true-positives by MRI. Among 18 patients with extrahepatic soft tissue metastases, 13 (72%) demonstrated targeting of soft tissue lesions on J591 scan.

Fourteen patients in these trials received multiple doses of radiolabeled J591: 10 patients received two doses and four patients received three doses. In 10 patients, repeat imaging studies were performed after each dose. In all of these cases, known sites of disease were present on conventional imaging studies. In every case, J591 targeting continued to be consistent with conventional scans on each sequential J591 imaging study. No evidence of more rapid clearance or increased reticuloendothelial uptake was seen.[29]

No patient in either trial has developed HAHA. The dose-limiting toxicity of myelosuppression in the ^{90}Y-HuJ591 study occurred at a dose of 20 mCi/m^2. The ^{177}Lu-huJ591 trial is ongoing with dose-limiting toxicity not yet reached. Dose-related antitumor activity has been noted in both trials, including both PSA and measurable disease responses. These studies will be reported in detail when patient accrual and analyses are completed in the near future.

Cytotoxin-conjugated J591. The first phase I trial of DM1-J591 has begun to explore single ascending doses of the conjugate to define the dose-limiting toxicity, MTD, and pharmacokinetics. A subsequent phase I trial of multiple-dose DM1-J591 is expected to open in mid-2003.

Clinical trials in solid tumor malignancies using J591. In addition to prostate epithelial cells, immunohistochemical studies show that PSMA is also expressed by vascular endothelial cells of numerous solid tumor malignancies, but not by normal vascular endothelium or in neoplastic epithelial cells of nonprostate malignancies.[18,30] PSMA expression by tumor-associated neovasculature was confirmed by CD34 double immunostaining.[30] A subsequent analysis of PSMA expression using reverse-transcription polymerase chain reaction and in-situ hybridization similarly demonstrated mRNA transcripts for PSMA in the endothelium of tumor-associated neovasculature of multiple nonprostatic solid tumor malignancies.[31] These data suggest that PSMA may be an effective target for mAb-based vasculotoxic therapy. Therefore, we initiated a phase I dose-escalation trial of ^{111}In-

labeled mAb huJ591 to test the hypothesis that huJ591 could target the neovasculature in nonprostate solid tumors; to define the huJ591 MTD and toxicity in non–prostate cancer patients; to determine the pharmacokinetics and biodistribution of huJ591; and to assay for the development of HAHA. Eligible patients included those with refractory solid tumor malignancies. Patients with a variety of solid tumors have been entered, including renal, bladder, colon, pancreatic, breast, and lung cancers. Similar to patients with prostate cancer, mAb huJ591 was very well tolerated with no development of HAHA. ^{111}In-J591 scanning showed localization of J591 to tumor sites in 15 of 19 patients.[32] Localization of J591 occurred in metastatic sites in viscera, soft tissue, and bone.

CONCLUSION

PSMA represents an ideal cell surface protein for targeted therapy of prostate cancer and vasculotoxic therapy of nonprostate solid cancers. Clinical trials using mAb J591, which recognizes the extracellular domain of PSMA, indicate that this antibody can effectively target disseminated prostate and non–prostate cancers in patients. Furthermore, J591 can be used to deliver radioisotopes or other cytotoxins to these cancer sites. Major, objective responses have been seen in the phase I prostate cancer trials. Ongoing and future clinical trials will define the therapeutic role of J591.

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Original Articles

TARGETING METASTATIC PROSTATE CANCER WITH RADIOLABELED MONOCLONAL ANTIBODY J591 TO THE EXTRACELLULAR DOMAIN OF PROSTATE SPECIFIC MEMBRANE ANTIGEN

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ABSTRACT

Purpose: We performed an interim analysis of imaging data collected in 2 phase I radioimmunotherapy trials to determine the ability of monoclonal antibody (mAb) J591 directed to the extracellular domain of prostate specific membrane antigen (PSMA) to target sites of known metastatic prostate cancer accurately.

Materials and Methods: Patients with progressing hormone independent prostate cancer were entered in 2 phase I dose finding trials with radiolabeled mAb J591. J591 is the first mAb targeting the extracellular domain of PSMA as well as the first de-immunized (humanized) mAb to PSMA to be tested in humans. These trials were primarily designed to assess dose limiting toxicity, maximum tolerated dose, pharmacokinetics and organ dosimetry. Planar gamma camera imaging studies obtained on the first 53 patients were reviewed and compared to sites of metastatic prostate cancer visualized on conventional imaging studies including bone scan, computerized tomography and/or magnetic resonance imaging. In 1 trial 29 patients received ^{111}indium-J591 for imaging followed by ^{90}yttrium-J591 for therapy. In the parallel trial 24 patients were treated with ^{177}lutetium-J591, an isotope that can be imaged directly.

Results: Of 53 patients reviewed 46 (87%) had evidence of metastatic disease on conventional scans. Overall, of the 43 evaluable patients J591 accurately targeted bone and/or soft tissue lesions in 42 (98%). J591 accurately targeted bone lesions in 32 of 34 (94%) and soft tissue lesions in 13 of 18 (72%) evaluable patients.

Conclusions: Radiolabeled J591 accurately targets bone and soft tissue metastatic prostate cancer sites, and may be useful for targeting therapeutic and/or diagnostic imaging agents.

KEY WORDS: prostatic neoplasms; antibodies, monoclonal; radioimmunodetection, neoplasm metastasis

Between 30% and 50% of patients who undergo treatment for clinically localized prostate cancer later manifest signs of systemic disease.[1] An additional 25% of newly diagnosed cases have evidence of regional or distant disease at initial diagnosis.[2] These figures yield an incidence of approximately 100,000 American men diagnosed with disseminated prostate cancer annually. Median survival for patients with metastatic, hormone refractory disease is 12 to 18 months.[3,4] Clearly we need to develop improved systemic therapies that, ideally, are applicable to the full spectrum of disease ranging from micrometastatic disease at early diagnosis to overt metastatic disease.

In recent years targeted therapy using monoclonal antibodies (mAbs) directed to cancer related cell surface antigens has been clinically validated. Since 1997 the Food and Drug Administration has approved several mAbs for treatment of various cancers as well as nonmalignant diseases. The most direct means of confirming in vivo tumor specificity is the administration of radiolabeled antibody to patients with known sites of disease to evaluate antibody targeting by imaging studies.

In prostate cancer the most well established, prostate restricted cell surface antigen yet identified is prostate specific membrane antigen (PSMA).[5–10] PSMA is an ideal target since it is expressed by all prostate cancers,[7,8,11–13] and expression levels increase progressively in more poorly differentiated, metastatic and hormone refractory cancers.[7,8,12,13] The first antibody to PSMA (7E11) was tested in vivo and

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† Financial interest and/or other relationship with BZL Biologics, Inc.

***Editor's Note:* This article is the first of 5 published in this issue for which category 1 CME credits can be earned. Instructions for obtaining credits are given with the questions on pages 1982 and 1983.**

later commercialized as an imaging agent (capromab pendetide). In vivo studies demonstrated that capromab could target known sites of soft tissue metastases in approximately two-thirds of patients.[14] However, capromab did not satisfactorily target bone metastases,[14,15] the most common site of metastatic disease, thus explaining why the agent is not approved for imaging bone metastases. Capromab can target soft tissue but not bone metastases because it recognizes an intracytoplasmic site of the PSMA molecule.[16,17] In viable cells the capromab binding site is masked by the intact cell membrane and is "invisible" to circulating antibody. The ability of capromab to target soft tissue sites has been proposed to be due to the presence of dead or dying cells with disrupted cell membranes. This hypothesis was recently proven by in vivo localization studies by Smith-Jones et al that demonstrated capromab localized in vivo only to areas of necrotic prostate cancer.[18] Bone marrow metastases, unlike soft tissue metastases, tend to be small, well vascularized lesions without necrosis, which explains the failure to be targeted by capromab. It had been hypothesized that an antibody to the extracellular domain of PSMA would result in improved targeting due to such an antibody's ability to "see" the large amount of PSMA displayed on the prostate cancer cell's exterior and its related ability to bind to viable cells.[19]

We report the first clinical studies of mAb J591, the first mAb to the extracellular domain of PSMA to be tested in patients, and present the tumor targeting results of the first 53 patients to receive radiolabeled J591 in 2 independent, phase 1 trials. These trials were designed to look primarily at toxicity, pharmacokinetics and organ dosimetry of radiolabeled J591 as a first step in the development of a therapeutic agent, not to assess the efficacy of the antibody for diagnostic imaging. As a by-product of these trials we are able to report here the ability of the J591 antibody to target metastatic prostate cancer sites in vivo.

MATERIAL AND METHODS

Material. Murine J591 was de-immunized by Biovation, Ltd. (Aberdeen, Scotland).[20] Briefly, de-immunization involves removal of mouse amino acid sequences and replacement with homologous human, nonimmunogenic sequences. Clinical grade de-immunized J591 was produced at Lonza Biologics, plc. (Slough, United Kingdom) and subsequently covalently linked with the chelating agent DOTA by Goodwin Biotechnology, Inc. (Plantation, Florida). The DOTA moiety allows retention of radiometals such as ^{111}indium (^{111}In), ^{90}yttrium (^{90}Y) and ^{177}lutetium (^{177}Lu) by the antibody. J591-DOTA was provided by BZL Biologics, Inc. (Framingham, Massachusetts) under U. S. Food and Drug Administration Investigational New Drug Applications 9279 and 9638. ^{111}In and ^{90}Y were purchased from Nordion (Kanata, Ontario), and ^{177}Lu was purchased from the University of Missouri (St. Louis, Missouri). J591 was labeled with radioisotope in the Nuclear Medicine Pharmacy of New York-Presbyterian Hospital at a specific activity of 3 to 15 mCi/mg. Additional unconjugated ("cold") antibody was added to give a constant protein dose of 20 mg in the ^{111}In/^{90}Y-J591 trial, or 10 mg/m^2 in the ^{177}Lu-J591 trial. In the ^{111}In/^{90}Y-J591 trial patients received 5 mCi of ^{111}In-J591 for pharmacokinetic, biodistribution and dosimetry studies 1 week before ^{90}Y-J591 administration. In the ^{177}Lu-J591 trial patients received ^{177}Lu-J591 ranging from 10 to 70 mCi/m^2. All antibody infusions were given intravenously at an infusion rate of 5 mg or less per minute.

Patients. A total of 53 patients (table 1) have enrolled thus far in 2 independent phase I radioimmunotherapy trials with ^{111}In/^{90}Y-DOTA-J591 (29) or ^{177}Lu-DOTA-J591 (24 patients) and are the subjects of this interim targeting analysis. Patients with progressing metastatic or recurrent prostate cancer underwent disease staging with conventional imaging modalities including chest x-ray, radionuclide bone scan and computerized tomography (CT) of the abdomen and pelvis. Since prior studies have shown that virtually all prostate cancers are PSMA positive,[8,11,13] no histological confirmation was performed to determine PSMA expression.

TABLE 1. *Patient demographics*

	All Pts		^{111}In/^{90}Y		^{177}Lu	
Total No. pts	53		29		24	
Age (range)	67.8 (47–85)		68.8 (49–85)		66.7 (47–84)	
No. radical prostatectomy (%)	23	(44)	12	(41)	11	(46)
No. radiotherapy (%)	29	(55)	18	(62)	11	(46)
No. hormonal therapy	52		29		23	
No. cytotoxic chemotherapy (%)	19	(36)	12	(41)	7	(29)
No. bony metastases on bone scan (%)	34	(64)	19	(66)	15	(60)
No. soft tissue metastases on CT or MRI:	21	(40)	14	(48)	7	(29)
Lymph nodes	15	(28)	10	(35)	5	(21)
Hepatic	3	(6)	1	(3)	2	(8)
Adrenal	2	(4)	2	(7)		
Pulmonary	1	(2)	1	(3)		
Local/pelvic mass	4	(8)	3	(10)	1	(4)

Study design. Radioimmunoscintigraphy was performed within 1 hour after antibody infusion and at 3 to 4 additional time points within 7 or 14 days after ^{111}In-J591 or ^{177}Lu-J591 injection, respectively. Imaging was performed using a gamma camera with a medium energy collimator. When unexpected lesions were detected on J591 scan confirmatory conventional imaging such as bone scan, CT or magnetic resonance imaging (MRI) were then performed as indicated. A total of 14 patients received up to 3 doses of radiolabeled J591 at intervals ranging from 7 to 33 weeks (median 10). In 10 patients these doses were followed by repeat imaging. After doses 2 and 3 a single imaging study was done on day 5 or 6 for comparison to the first imaging study to determine whether radiolabeled antibody continued to localize to tumor sites.

Evaluation. Radioimmunoscintigraphic images were reviewed by attending nuclear radiologists (LK and SJG) and an attending urologist (NHB), and compared to conventional imaging studies. Soft tissue lesions 2 cm or larger in diameter on CT or MRI were presumed to represent metastatic cancer. Due to radioisotope excretion by the liver, hepatic lesions were not evaluable and, therefore, excluded from comparative evaluation. All areas of increased uptake on bone scan, with the exception of those sites involved with degenerative joint disease and/or arthritis, were presumed to represent bone metastases.

RESULTS

Of 53 patients in this series 46 (87%) had radiographic evidence of metastatic disease while 7 (13%) patients had no visible osseous or soft tissue lesions. A total of 34 patients (64%) had metastases demonstrated on radionuclide bone scans. A single patient with an increasing prostate specific antigen of 3.6 had a negative bone scan but sclerotic lesions on CT. The J591 scan of this patient was consistent with bone scan (ie negative), however, because of the inconsistency between bone and CT scans he was considered inevaluable for targeting assessment. Soft tissue lesions 2 cm or larger, defined as metastatic disease and demonstrated on CT, were found in 21 patients (40%). Three patients whose disease exclusively involved the liver were excluded from comparative evaluation with J591 imaging. Overall J591 accurately targeted bone and/or soft tissue lesions in 42 (98%) of the 43 evaluable patients (figs. 1 and 2). Of the 7 patients with neither osseous nor soft tissue lesions visible on conventional imaging, 4 had negative J591 scans. In the remaining 3 patients (43%) J591 imaging demonstrated unexpected met-

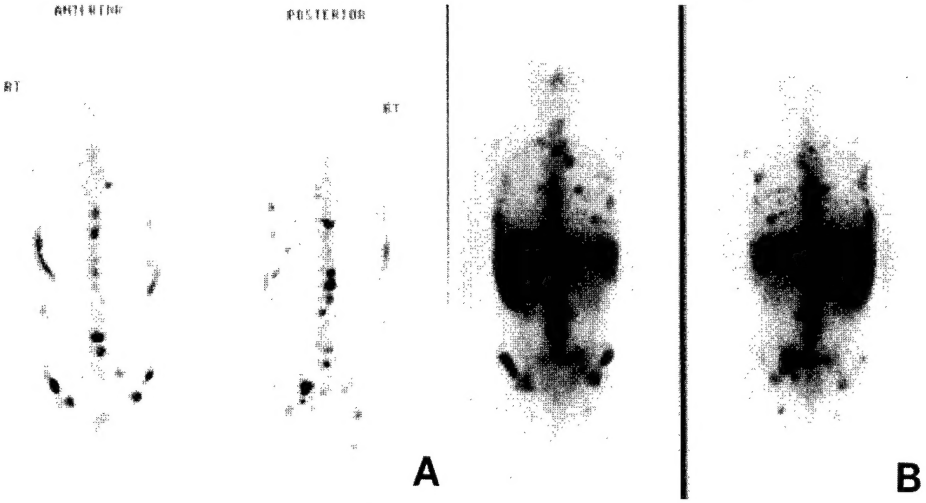

FIG. 1. Bone scan (*A*) and J591 scan (*B*) from same patient. Bone scan shows excretion through kidneys and bladder as well as multiple areas of increased uptake in ribs, spine and pelvis. J591 scan in addition to liver excretion of radiometal shows superimposable areas of J591 accumulation/targeting.

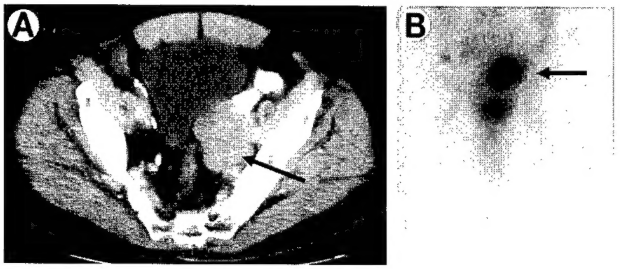

FIG. 2. CT (*A*) showing soft tissue mass measuring 4 × 6 cm in left pelvis. Anterior view of J591 scan (*B*) showing accumulation in left pelvic mass.

astatic lesions either in bone or soft tissue, all of which were confirmed by subsequent MRI or CT studies (fig. 3).

The targeting accuracy of J591 scans compared with conventional imaging stratified by disease site is shown in table 2. Of 34 patients with osseous lesions on bone scan, 32 (94%) demonstrated bone lesion targeting with J591, and 2 had false-negative antibody imaging results. Of 18 patients with no evidence of bone metastasis on conventional imaging, 16 (89%) had congruent results with J591 imaging. The 2 false-positive J591 scans were later confirmed to be true-positives by MRI.

In 18 patients with extrahepatic soft tissue metastases, 13 (72%) demonstrated targeting of soft tissue lesions on J591 scan. A total of 35 patients had no visible extrahepatic soft tissue metastases on conventional imaging, and 34 of these patients (97%) had negative J591 scans with 1 having a false-positive antibody scan. This false-positive J591 scan demonstrated a lesion in the superficial inguinal region.

In this series 29 patients had prostates in situ with the remainder having undergone radical prostatectomy. Of these 29 patients 14 underwent single photon emission CT of the pelvis. Only 1 of 14 had good visualization of the prostate while the remainder had either nonvisualization or poor visualization. The patient with good visualization had prior hormonal and chemotherapy but no radiotherapy, whereas the others had received radiotherapy to the prostate. None of these patients had clinical evidence of local progression and none underwent prostate biopsies as part of this study.

In these trials 14 patients received multiple doses of radiolabeled J591, 10 received 2 doses and 4 received 3 doses. In 10 patients repeat imaging studies were performed after each dose. In all of these cases known sites of disease were present on conventional imaging studies. In every case J591 targeting continued to be consistent with conventional scans on each sequential J591 imaging study. No evidence of more rapid clearance or increased reticuloendothelial uptake was seen (fig. 4).

DISCUSSION

The ability to target tumor sites specifically and accurately without targeting normal sites is the core principle of antibody mediated imaging and therapy. As such it seemed appropriate to assess the ability of the J591 antibody to target in vivo. We report on the first clinical study of J591, the first mAb to the extracellular domain of PSMA to be tested in patients. Although targeting the same molecule as capromab, J591 targets a different binding site of PSMA situated on the exterior of the cell, whereas capromab binds a site of PSMA within the interior of the cell. It has been demonstrated that an antibody to the extracellular domain of PSMA provides improved antibody targeting in vivo[18] because of the greater accessibility of the extracellular antigenic site and the related ability of the J591 antibody to bind viable cells, both features lacking in capromab. Since these phase I therapy trials predominately studied patients with metastases detectable on conventional imaging studies, they provided us with specific marker sites at which we could also assess antibody targeting.

Consistent with capromab and other proteins or peptides labeled with radiometals, we found isotope processing occurs predominately through the liver. In patients with soft tissue metastases J591 targeted successfully in 13 of 18 (72%) patients. In a few cases the J591 scan revealed additional sites of apparent nodal disease, but since these were neither visible on CT/MRI nor biopsied, these sites were not considered evaluable. By comparison capromab imaging has been evaluated and reported in 6 patients with clinically imaged soft tissue disease.[14] Capromab imaging was positive in 4 of the 6 (67%) patients. The limited data with capromab reflect the fact that most studies of this agent have entered patients without clinically evident disease.

Where the results of J591 and capromab appear to diverge substantially is in the targeting of bone metastasis. In the present study we found that J591 targeted bone metastases in 32 of 34 (94%) patients, and in virtually all instances the J591 scan was superimposable on the bone scan. J591 imaging was false-negative in only 2 patients. Furthermore, J591 detected unexpected osseous metastases in 2 patients with

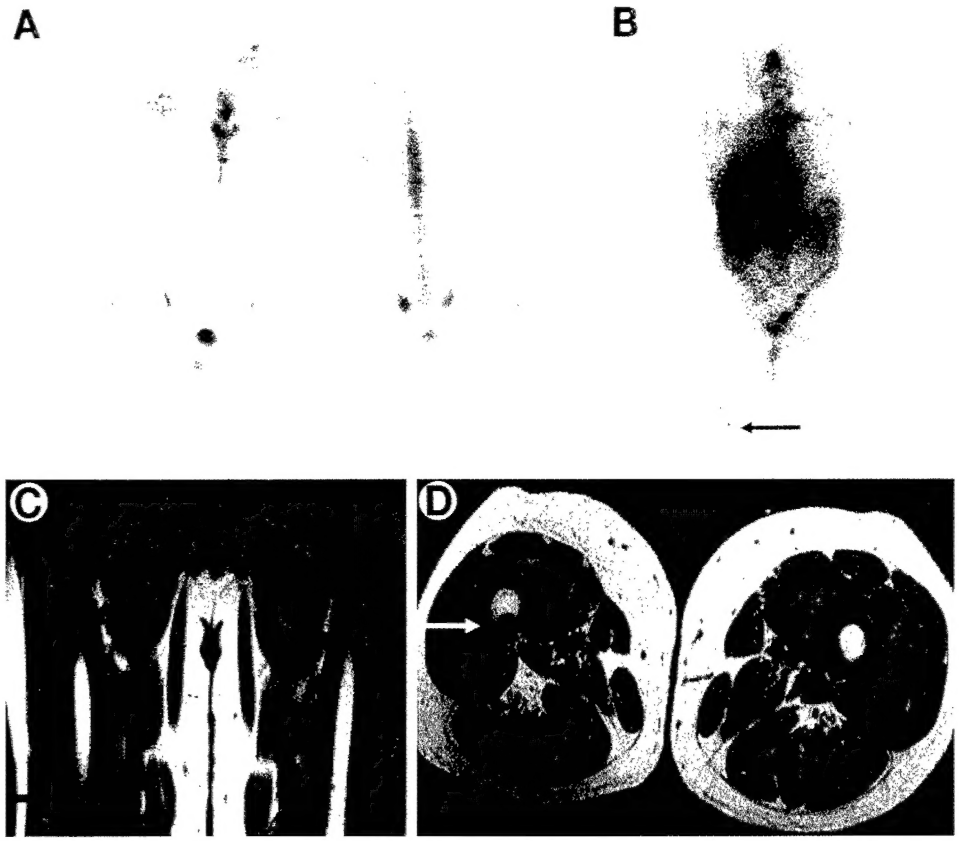

FIG. 3. Anterior and posterior views of bone scan (*A*) show no lesions. J591 scan (*B*) showed consistent focal uptake in right femur (arrow) that prompted MRI (*C* and *D*). MRI confirmed bone metastasis (arrows).

TABLE 2. *Targeting of J591 scans compared with conventional imaging*

	No./Total No. (%)		
	^{111}In	^{177}Lu	^{111}In + ^{177}Lu
Bony metastasis	17/19 (84)	15/15 (100)	32/34 (94)
Soft tissue metastasis (extrahepatic)	9/13 (69)	4/5 (80)	13/18 (72)
Bony and/or soft tissue metastasis	26/32 (81)	19/20 (95)	45/52 (87)

negative bone and CT scans that were later confirmed by conventional imaging. In the case of capromab, 2 published studies reported targeting results in patients with clinically evident bone metastases. Wynant et al from the Cytogen Corporation reported results in 38 patients with positive bone scans.[14] Only 5 of the 38 (13%) patients with positive bone scans had all their lesions detected, while 17 of the 38 (45%) were completely negative on capromab scan. Another study by Deb et al reported on 12 patients imaged with capromab.[15] Of 12 patients 11 had multiple (10 or more) lesions on bone scan. Similar to the results in the Wynant et al study, on capromab imaging 5 of these 11 (45%) patients had no bone lesions targeted at all. In none of the remaining patients with multiple lesions did capromab target more than 1 of the lesions.

In our series 29 patients had intact prostates and 14 underwent analysis of single photon emission CT of the prostate after ^{111}In-J591. In general the prostate was not well visualized on J591 imaging in these patients despite excellent visualization of metastatic disease. None of these patients with intact prostates had clinical evidence of local progression and none underwent prostate biopsy as part of this study. Possible reasons for absent prostate targeting include the absence of local prostate cancer after local radiotherapy, hormonal therapy and in some cases, chemotherapy, and PSMA expression in residual non-neoplastic prostate epithelium being predominately in the form of PSM′. PSM′ is a splice variant of PSMA expressed in cytoplasm but not the cell membrane. Interestingly the 1 patient whose prostate was well visualized was the only patient of the 14 who did not have prior radiotherapy. Ideally evaluation of antibody targeting of primary prostate cancer should occur in patients who have not received such treatments previously and should include histological assessment of the prostate.

None of the patients in this study underwent biopsy before entry to determine PSMA expression. That virtually all patients targeted successfully with J591 provide in vivo confirmation of pathology studies finding that virtually all prostate cancers are PSMA positive.[7,8,11–13]

In this series 10 patients received multiple doses of radiolabeled J591 followed by repeat imaging. These studies revealed persistent tumor targeting (fig. 4) indicating continued expression of PSMA without selection of PSMA negative clones, no immune response to J591, and the ability to target tumor sites repeatedly for imaging and/or treatment.

PSMA is expressed at much lower levels in kidney and small bowel relative to prostate cancer. Yet no targeting to these organs was seen. The lack of targeting of these organs likely relates to the substantially lower level of expression (1/100 to 1/1,000)[10] as well as to the luminal sites of expression in these tissues, beyond the basement membrane and tight junctions, effectively ex vivo, where the intact antibody has little or no access.

CONCLUSIONS

We have demonstrated that J591, an antibody to the extracellular domain of PSMA, is able to target sites of metastatic prostate cancer sensitively and specifically in bone and soft tissue. The antibody is nonimmunogenic and can be administered multiple times to the same patient with persis-

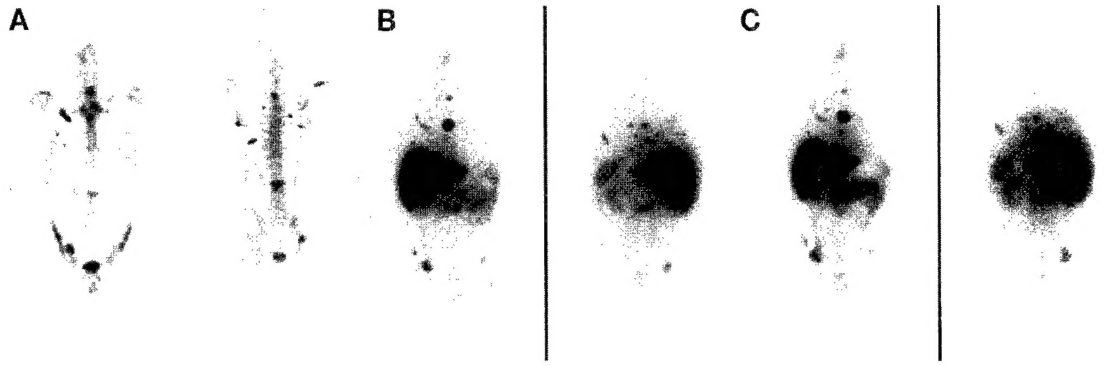

FIG. 4. Bone scan (*A*) and J591 images (*B* and *C*) from patient who received 2 doses of ^{177}Lu-J591 (5 days after 1st dose [*B*] and 6 days after 2nd dose of antibody [*C*]). Dose 2 was given 3 months after dose 1. Scans are virtually superimposable, demonstrating persistent expression of PSMA and continued ability to localize to tumor sites.

tently accurate targeting. J591 shows promise as an in vivo targeting agent with potential diagnostic imaging and therapeutic usefulness. The ability of J591 to image not only soft tissue but also bone metastases consistently confirms that targeting of the extracellular domain of PSMA and the related ability to bind viable cells make it a better in vivo targeting agent than capromab. The proven targeting ability of J591 supports use in therapy as a tumor targeting mAb vehicle. In unmodified form J591 could focus the immune system on tumor sites. Alternatively, J591 could serve as a tumor targeting mAb vehicle to deliver cytotoxic radioisotopes and/or drugs to tumor sites without adverse effects on nontargeted normal tissues.

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Dr. Neil H. Bander developed the J591 antibody used in this study and he served as Principal Investigator of the trials reported here. J591 and related anti-PSMA extracellular domain antibody patents were assigned to the Cornell Research Foundation and subsequently licensed to BZL Biologics, Inc.

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